The development of drugs based on ergot alkaloids (EA) was probably the primary driving force leading to the first attempts at the standardization of ergot preparations in the second half of the 19th century. Elemental analysis, titration, melting point, optical rotation, some color reactions, various physiological experiments and precipitation tests were the only guides used for this purpose practically till the thirties of the 20th century (Evers, 1927). Standardization of ergot preparations, and particularly the monitoring of some degradation processes were the main objectives of the analytical chemistry of EA between the wars (for a review see Swanson et al., 1932). The first colorimetric methods introduced by Evers (1927) and van Urk (1929) can be considered a milestone in the analytical chemistry of EA. Since these procedures are limited to the determination of the total content of EA only, regardless of their activity, bioassays dominated the field until the fifties, when the first quantitative chromatographic methods appeared (Fuchs, 1953; Gyenes and Bayer, 1961). The development of planar chromatographic techniques after the World War II, hand in hand with the general improvement of separation techniques, led to the isolation and description of the majority of EA in the fifties and sixties, as well as to the discovery of the majority of reactions responsible for their instability. High performance liquid chromatography was introduced to the analysis of EA in the early seventies. Almost simultaneously, immunological methods were developed as a convenient alternative to the chromatographic methods (Van Vunakis et al., 1971; Taunton Rigby et al., 1973; Castro et al., 1973; Loeffler and Pierce, 1973). Sophisticated TLC and HPLC methods enabling the distinguishing and quantitative analysis of all common EA have been described in the eighties; with some minor modifications, and with much better chromatographic material quality, they are still in use. These procedures are nowadays supplemented also by modern coupled chromatographic-mass spectrometric methods.

10.1. COLORIMETRY, SPECTROPHOTOMETRY, AND FLUORIMETRY

The first colorimetric test based on the reaction of EA with concentrated sulphuric acid was described by Tanret as early as 1875. Many slight modifications of this procedure have appeared since then and a careful survey
of the literature would indicate that this reaction has been “rediscovered” several times. The first semiquantitative analytical method for the determination of EA developed by Evers (1927) uses the same principle. Two years later, van Urk (1929) described the reaction of EA with $p$-dimethylaminobenzaldehyde and its use for quantitative analysis. This method has undergone many modifications (for a review see Gyenes and Bayer, 1961; Michelon and Kelleher, 1963; Lászlóne and Gábor, 1967), but its principle has remained practically unchanged. Spectrophotometric assay with $p$-dimethylaminobenzaldehyde reagent has been employed as an official assay for the determination of EA according to the European Pharmacopeia (1997), and as a fast in-process quantification of EA produced by submerged fermentation. This reagent is also a key component of the Ehrlich reagent used for visualisation of EA on TLC plates.

An exposure to the light was necessary for the full color development according to the procedure by van Urk (1929). Irradiation could be eliminated by adding a small amount of ferric chloride to the reagent (Allport and Cocking, 1932) and/or these two factors could be combined. The third principal modification was introduced by Sprince (1960) who had noticed that an additional spraying of paper chromatograms developed with the $p$-dimethylaminobenzaldehyde reagent with sodium nitrite considerably reduced the time necessary for the development as well as the tendency of the spots to fade. Similarly as with other indoles, two molecules of an ergot derivative condens with one molecule of $p$-dimethylaminobenzaldehyde in the acidic media to produce an intense blue color. 2-Substitued-derivatives create red color, but 2,3-disubstituted analogues do not react at all (Pöhm, 1953). Comparison of various Spectrophotometric methods has been published by Michelon and Kelleher (1963). Depending on the particular modification of the method, slightly different UV spectra of the product(s) could be obtained resulting in a different slope of the calibration curves. Since the reaction conditions must be carefully followed to avoid the formation of the colored byproducts, a Spectrophotometric determination of EA with ninhydrin has been proposed recently as an alternative method (Zakhari et al., 1991).

A number of EA exhibit a strong fluorescence upon irradiation. This feature is extensively used for the detection of EA after their separation by HPLC or TLC. Without any preceding separation, the fluorescence of ergot derivatives can also be used for the development of some fluorimetric assays (Gyenes and Bayer, 1961; Hooper et al., 1974). The limitation of all direct methods of EA determination including colorimetry, spectrophotometry or fluorimetry is the lack of specificity regarding related alkaloids or their own degradation products. Consequently, the majority of these methods has now been abandoned, but the specific features or reactions of EA are further employed for their detection by more sophisticated HPLC and TLC methods.

In addition to various methods employing the absorbance or fluorescence of EA in the UV-vis or near infrared region, reflectance spectroscopy was
examined as a possible alternative to HPLC analysis for ergovaline in tall fescue (Roberts et al., 1997). In contrast to the above mentioned methods permitting EA determination only in rather simple matrices, NIR spectroscopy is a nondestructive technique making it possible to rapidly process a large number of whole-tissue samples. In spite of the precision and detection limit achieved (hundreds of µg of ergovaline per kilogram of tall fescue), the narrow range of samples examined so far will have to be substantially expanded to obtain more general evaluation of this method in the future. However, with regard to obvious problems with the validation of this procedure, it cannot be expected that NIR spectroscopy might replace current methods such as HPLC or immunoassays.

10.2. PLANAR CHROMATOGRAPHY

Interesting circular paper chromatographic methods were described by Berg (1951, 1952), but they were soon replaced by procedures based on descending paper chromatography enabling the exact localization and extraction of individual chromatographic spots. Buffers and solvent mixtures such as butanol-acetic acid-water (organic layer) or aqueous phase of the same mixture for the paper impregnated with silicon were used in the original methods (Tyler and Schwarting, 1952). Since the impregnation of paper with formamide provided better and more reproducible resolution for the majority of alkaloids, this procedure has later become an analytical standard (Tyler and Schwarting, 1952; Stoll and Rüegger, 1954; Macek et al., 1954, 1956; Horák and Kudrnáč, 1956; Klošek, 1956a; Pöhm, 1958). Since some alkaloids were still not resolved, their mutual ratio was determined by the amino acid and keto acid analysis of their acid or alkaline hydrolysates, respectively (Stoll and Rüegger, 1954; Pöhm, 1958). Extraction of individual spots in combination with the reaction with p-dimethylaminobenzaldehyde enabled the development of the first quantitative colorimetric or photometric assays for the determination of individual EA (Klošek, 1956b; Pöhm, 1958). A paper chromatographic method has been described also for LSD (Faed and McLeod, 1973) and clavine alkaloids using benzene-chloroform-ethanol or butanol-water mixtures (Kornhauser et al., 1970).

More recently, paper chromatography and thin layer chromatography were used to analyze the EA produced by the toxic plant Ipomoea muelleri (Marderosian et al., 1974). The time necessary for one analysis, poor resolution as well as low reproducibility caused paper-chromatographic methods to be quickly and almost completely replaced by thin-layer chromatography (TLC) on silica gel in the sixties; now they have a merely historical significance.

The thin layer chromatography of EA was first described about 1958, and since then it has become one of the most important methods for the quantitative and qualitative analysis of this group of compounds. It is worth mentioning that TLC is also the official method for the analysis of related alkaloids described
in the current European Pharmacopeia (1997). Among several tens of the literature references, the majority of separations were carried out on common silica gel. The use of aluminium oxide or even chalk was also described (for a review see Reichelt and Kudrnáč, 1973). Separation on cellulose plates was reported as an improvement of the older paper chromatographic techniques (Teichert et al., 1960; Fowler et al., 1972; Prošek et al., 1976a, b) but, generally, none of these materials seems to offer any advantage in resolution when compared with a separation on silica gel. Some representative methods for various classes of EA are summarized in Table 1. Among many published methods, the following papers can be particularly recommended for clavines: Klavehn and Rochelmeyer (1961), Marderosian (1974), Wilkinson et al. (1987); for ergopeptines: Fowler et al. (1972), Reichelt and Kudrnáč (1973), Bianchi et al. (1982), and Crespi-Perellino et al. (1993).

All natural EA are colorless, the slightly yellow or even greenish color in some preparations being caused by some degradation processes on exposure to air and/or light. Hence, some appropriate reaction must be used to visualize the TLC spots to enable their examination by naked eye. In the simplest way the spots can be developed by iodine vapours to produce a transient yellow color. Van Urk’s color reaction, or its numerous modifications (including also Ehrlich’s reagent: p-dimethylaminobenzaldehyde-conc. HC1), are undoubtedly the most popular methods, described in various pharmacopeias. Some limitations of this method have already been mentioned in the part on colorimetry. People engaged in environmental analysis and new compound hunters would surely appreciate the subtle color differences accompanying slight structural changes, e.g., epimerization at C8 or the presence of benzyl group in the peptide moiety. Similarly, various colors ranging from blue, azure, green, pink to yellow, were used to indicate the structural modifications and presence of some functional groups in the clavine series (Yamatodani, 1960; Klaven and Rochelmeyer, 1961; Křen et al., 1986, 1996b). However, the proper interpretation of these differences demands a previous experience. The main advantage of this method is the stability of the majority of colors that enables one to store the original plates for years as documentation material. Besides the native fluorescence of some EA derivatives, the reactions with perchloric acid-FeCl3 reagent or with the Dragendorff reagent can be alternatively used to visualize these spots (Keiper and Voigt, 1972). EA can also be visualized by charring with 50% sulphuric acid at 110°C for 10 min (Scott and Kennedy, 1976). Alternatively, a procedure with 5–10% sulphuric acid in ethanol provides a better estimation of individual EA and more specific indication of unrelated impurities (Křen, private communication). The π-complex formation of aromatic rings in EA with some π-acceptors, e.g., 7, 7, 8, 8-tetracyanoquinodimethane, 2, 4, 7-trinitrofluorenone or 2, 4, 5, 7-tetranitrofluorenone was also tested (Rücker and Taha, 1977), but neither of these nor other reagents described in the literature have found a widespread use.
After the separation, EA can be quantitatively analyzed either directly “in situ”, or indirectly, by scraping off the spots, eluting the active substances and by some subsequent qualitative or quantitative detection. The original procedures used extraction of individual spots and photometric or fluorimetric determination of the alkaloids (Dal Cortivo et al., 1966; Keipert and Voigt, 1972). Although this method could be used also with the current commercially available TLC plates (Wilkinson et al., 1987), it is very cumbersome, particularly in the case of multicomponent analysis. Hence, it is used now for the off-line identification of unknown components rather than for quantitative analysis. Ergot alkaloid mixtures can be quantitatively analysed by TLC—densitometry, e.g., after the reaction with the van Urk reagent (Genest, 1965). To avoid any inhomogeneity caused by spraying, \(p\)-dimethylaminobenzaldehyde can also be directly added to the solvent system and the development is accomplished in HC1 vapours. Alternatively, EA might be quantitatively analysed with higher sensitivity also by TLC—fluorodensitometry (Niwaguchi and Inoue, 1971; Eich and Schunack, 1975; Prošek et al., 1976a, b; Nelson and Foltz, 1992a). However, it should be noted, that some alkaloids or their semisynthetic derivatives do not exhibit fluorescence (Gröger and Erge, 1963).

10.3. GAS CHROMATOGRAPHY

Some low molecular weight clavine alkaloids and simple lysergic acid amides lacking hydrophilic functional groups could be directly separated on a gas chromatographic column (Radecka and Nigam, 1966; Agurel and Ohlsson, 1971; Nichols et al., 1983; Japp et al., 1987; Clark, 1989; Flieger et al., 1991). Several derivatization reactions have been tested in order to overcome the low volatility of the alkaloids and to reduce the peak tailing. Silylation of clavines was found to produce single derivatives (Barrow and Quigley, 1975), but the silylation at the N1-atom is incomplete even when using a high excess of the silylation reagent and thus cannot be used for quantitative analysis (Agurel and Ohlsson, 1971; Křen and Sedmera, 1996a). Trifluoroacetylation of clavines was found to be unsuitable due to the formation of multiple derivatives and laborious reaction conditions (Barrow and Quigley, 1975).

The continued use of lysergic acid diethylamide (LSD), whose simple production, low cost and wide availability cause the increase of its popularity among drug addicts, has stimulated also efforts to develop effective analytical methods for its determination in body fluids. Among other methods currently used (fluorimetry, TLC, HPLC and immunoassays), gas chromatography represents a method of choice (for a review see Nelson and Foltz, 1992a). Several derivatization strategies have been successfully applied using either silylation with bis(trimethylsilyl)trifluoroacetamide (BSTFA) (Paul et al., 1990; Bukowski and Eaton, 1993), trifluoroacetylation at N1 with trifluoroacetylimidazole in
Table 1 Selected multicomponent TLC methods for the determination of EA

<table>
<thead>
<tr>
<th>Alkaloid class</th>
<th>Method†</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ergopeptines</td>
<td>silica gel G (benzene : DMF) silica gel G (AcOEt : DMF : EtOH) silica gel Al₂O₃, cellulose² silica gel H (CHCl₃ : MeOH) silica gel impregnated with DMF (DIPE : THF : toluene : DEA) silica gel G (AcOEt : DMF : EtOH) silica gel 60 (CHCl₃ : isopropanol) silica gel (CH₂Cl₂ : isopropanol)</td>
<td>McLaughlin et al., 1964 McLaughlin et al., 1964 Fowler et al., 1972² Vanhaelen and Vanhaelen-Fastré, 1972 Reichelt and Kudrnáč, 1973</td>
</tr>
<tr>
<td>Dihydroergopeptines</td>
<td>cellulose (AcOEt : heptane : DEA)</td>
<td>Prošek et al., 1976a, b</td>
</tr>
<tr>
<td>LSD</td>
<td>silica gel (trichloroethane : MeOH) silica gel G (MeOH : CHCl₃ : hexane) silica gel G (acetone : CHCl₃) silica gel G (MeOH : CHCl₃)</td>
<td>Dal Cortivo et al., 1966 Niwaguchi and Inoue, 1971</td>
</tr>
<tr>
<td>Lisuride maleate</td>
<td>silica gel (CHCl₃ : MeOH)</td>
<td>Clark, 1989 Amin, 1987</td>
</tr>
<tr>
<td>Nicergoline</td>
<td>silica gel (CHCl₃ : acetone : water)</td>
<td>Banno et al., 1991b</td>
</tr>
</tbody>
</table>

* Abbreviations: AcOEt—ethyl acetate, EtOH—ethanol, MeOH—methanol, DEA—diethylamine, DMF—dimethylformamide, DIPE—diisopropyl ether. † Fourteen alkaloids in eighteen solvent systems.
the presence of 1, 4-dimethylpiperazine (Lim et al., 1988), or treatment with a mixture of trimethylsilylimidazole, bis(trimethylsilyl)acetamine, and trimethylchlorosilane (3:3:2, v/v/v) (Nakahara et al., 1996). Derivatives can be analysed by conventional capillary columns, but serious attention has to be paid to the fact that the silyl-derivative is moisture sensitive and LSD exhibits a strong tendency to undergo irreversible adsorption on the glass during the chromatographic process (Paul et al., 1990; Nelson and Foltz, 1992a; White et al., 1997). Nowadays, a confirmation of LSD and its isomer lysergic acid N-methyl, N-<i>n</i>-propylamide (LAMPA), which is also a controlled drug, is usually performed by a combination of some chromatographic and mass spectrometric techniques that make it possible to distinguish these compounds (Nichols et al., 1983; Paul et al., 1990; Nelson et al., 1992a,b; White et al., 1997).

Because of their thermal instability, low vapor pressure, and potential occurrence of isomerization and/or decomposition products direct measurement of ergopeptine molecules by gas chromatography had been found unsuitable. In the first attempt, whole blood sample containing an ergot alkaloid was partly hydrolyzed with 6 N HC1 and characteristic decomposition products were analysed by GC (de Zeeuv et al., 1978). However, partial hydrolysis leads to irreproducible formation of several products and thus cannot be used for quantitative analysis. Nevertheless, total hydrolysis followed by the GC/MS analysis was used for the indirect determination of some ergopeptines containing unusual amino acids (Jegorov et al., 1997). Without the preceding hydrolysis, several direct methods based on the quantitative thermal decomposition of ergopeptines in the injection port of a gas chromatograph and the GC analysis of the decomposition products have been developed (Szepesi and Gazdag, 1976; van Mansvelt et al., 1978; Plomp et al., 1978; Larsen et al., 1979; Feng et al., 1992). The injector temperature is the key factor influencing the decomposition of ergopeptines. Initially, ergopeptines are split into lysergic acid amide (or corresponding dihydrolysergic acid amide) and a fragment derived from the tripeptide moiety. Several subsequent reactions can occur and the fragmentation is similar to that observed under the electron impact conditions in the mass spectra. In general, the reproducibility and accuracy of these methods is slightly lower than that of some HPLC methods, but they can be advantageously used in toxicological screening (van Mansvelt et al., 1978; Uboh et al., 1995). It should be noted that the annotation of these methods as “GC methods for ergopeptide alkaloids” in the literature, cited also by many authors, is slightly misleading as all these methods are in fact based on mass fragmentography (MF/GC).

10.4. LIQUID CHROMATOGRAPHY

First attempts to separate EA by liquid chromatography were made on unmodified silica gel (NP-HPLC) with mixtures such as diisopropyl ether-acetonitrile, chloroform-methanol, chloroform-acetonitrile or diethyl ether-methanol (Heacock
Separation of EA on silica gel provides an excellent resolution of individual EA groups in the elution order ergopeptinines > ergopeptines > ergometrine and clavines, which is very similar to that on TLC plates, and is also employed in the industrial purification of EA. Individual series of ergopeptines are resolved partially, i.e., ergotamines, ergoxines, and ergotoxines, but their individual members usually coelute (Szepesy et al., 1978, 1980; Zor et al., 1985). Since analytical methods based on “normal-phase” chromatography suffered from serious disadvantages (low resolution, peak tailing, strong adsorption of polar constituents), methods using unmodified silica were soon abandoned and replaced by liquid chromatography on various modified phases. Besides the use of silica in the “normal phase” mode, an interesting separation of lysergic acid, lysergamide, LSD and iso-LSD in the “reversed-phase” mode using methanol—0.2 N aqueous ammonium nitrate (3:2, v/v) is worth mentioning (Jane, 1975).

Separation of EA by reversed-phase liquid chromatography (RP-HPLC) was developed in the seventies and eighties. Nowadays all, or almost all, routine analyses are carried out on C-8 or C-18 columns. Similarly, some other modified silicas, e.g., phenyl (Otero et al., 1993; Cvak et al., 1994) or cyanopropyl (Sochor et al., 1995) can be used in the reversed-phase mode. Ergot alkaloids are eluted according to their increasing hydrophobicity, i.e., in the order: clavines < lysergic acid amides < ergopeptines < ergopeptinines, and for the particular series of ergotoxines in the order: ergocornine < β-ergokryptine = α-ergokryptine < ergocristine < ergogaline. Ergopeptinines (8-epimers) are always eluted later than the corresponding ergopeptines. Études on HPLC methods were described by many authors on columns from various producers and some examples of problems which could be solved by HPLC are summarized in Table 2. An additional information can be found also in three excellent reviews summarizing various aspects of EA analytical chemistry (Nelson and Foltz, 1992a; Garner et al., 1993; Flieger et al., 1997). In all HPLC methods, methanol-water or acetonitrile-water adjusted usually to slightly alkaline pH (ammonium or triethylamine buffers) were used as eluents. At alkaline pH, EA are separated as free bases providing usually much better selectivity compared with chromatography in “ion-pair” mode at low pH. Since ergopeptines are hydrophobic, water should be avoided for dissolution of samples due to their limited solubility and possible adsorption in injector loops (Otero et al., 1993). Most frequently, the detection of EA was accomplished by measuring the UV absorbance (usual detector setting to 310 nm for ergopeptines and 280 nm for dihydroergopeptines). A higher sensitivity and selectivity was achieved by fluorescence monitoring, e.g., with ergopeptines (Edlund, 1981; Cieri, 1987; Rottinghaus et al., 1991; Cox et al., 1992; Hill et al., 1993; Miles et al., 1996; Shelby and Flieger, 1997), dihydroergopeptines (Zorz et al., 1983; Zecca et al., 1983; Humbert et al., 1987), lysergic acid amides or simple ergolene derivatives (Cox et al., 1992; Nelson and Foltz, 1992a; de Groot et al., 1993; Wolthers et al., 1993; Brooks et al., 1997). The detector wavelength combination: excitation at 310 nm and
emission at 415 nm, is recommended for natural ergopeptines (Shelby and Flieger, 1997). However, it is important to note that the fluorescence intensity and position of maximum in the emission spectrum of alkaloids largely depends on the pH and solvent used (Hooper et al., 1974).

Table 2 Selected problems solved by RP-HPLC methods

<table>
<thead>
<tr>
<th>Alkaloid class</th>
<th>Matrix</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clavines and simple lysergic acid amides</td>
<td>ergometrine in pharm. preparations</td>
<td>Sondack, 1978; Tokunaga et al., 1983</td>
</tr>
<tr>
<td></td>
<td>lysergic acid and simple amides</td>
<td>Gill and Key, 1985; Flieger et al., 1993</td>
</tr>
<tr>
<td></td>
<td>clavine glycosides</td>
<td>Flieger et al., 1989, 1990; Kren et al., 1990</td>
</tr>
<tr>
<td></td>
<td>ergometrine in plasma</td>
<td>Edlund, 1981; Cox et al., 1992</td>
</tr>
<tr>
<td></td>
<td>ergometrine pharmacokinetics</td>
<td>de Groot et al., 1993</td>
</tr>
<tr>
<td></td>
<td>clavines produced by <em>Penicillium</em></td>
<td>Zelenkova et al., 1996</td>
</tr>
<tr>
<td>Ergopeptides</td>
<td>ergotamine preparations</td>
<td>Bethke et al., 1976; Erni et al., 1976; Cieri, 1987</td>
</tr>
<tr>
<td></td>
<td>various systems and columns</td>
<td>Dolinar, 1977</td>
</tr>
<tr>
<td></td>
<td>EA in wheat, flour, bread, pancakes</td>
<td>Scott and Lawrence, 1980, 1982; Scott et al., 1992; Fajardo et al., 1995</td>
</tr>
<tr>
<td></td>
<td><em>z, b</em>-ergokryptine distinguishing stability of alkaloids in sclerotia eight ergopeptines and their 8-epimers</td>
<td>Herényi and Görög, 1982; Young et al., 1983; Magg and Ballschmiter, 1985</td>
</tr>
<tr>
<td></td>
<td>in vitro ruminal digestion of ergovaline</td>
<td>Moyer et al., 1993</td>
</tr>
<tr>
<td></td>
<td>EA in blood</td>
<td>Moubarak et al., 1996</td>
</tr>
<tr>
<td></td>
<td>EA in tall fescue</td>
<td>Rottinghaus et al., 1991; Hill et al., 1993; Miles et al., 1996; Shelby and Flieger, 1997</td>
</tr>
<tr>
<td>Dihydroergopeptines</td>
<td>dihydroergotoxines in plasma</td>
<td>Zorž et al., 1983; Zecca et al., 1983</td>
</tr>
<tr>
<td></td>
<td>dihydroergotoxine components</td>
<td>Hartmann et al., 1978; Chervet and Plas, 1984; Papp, 1990</td>
</tr>
<tr>
<td></td>
<td>dihydroergotamine</td>
<td>Humbert et al., 1987; Niazi et al., 1988</td>
</tr>
</tbody>
</table>
The main area of use of HPLC methods is the control of impurities in bulk pharmaceutical substances (according to current general rules, all impurities above 0.1 rel % are analyzed and declared), and control of final dosage forms (stabilities, accelerated stress tests). Particular attention is paid to the stability of essential drugs during the shipment to the tropics, e.g., of ergometrine and methylergometrine maleates (Hogerzeil et al., 1992; Hogerzeil and Walker, 1996). Increasing attention is devoted also to the determination of the metabolic fate of EA. Studies of the metabolism of bromokryptine (Maurer et al., 1982, 1983) and dihydroergotamine (Maurer and Frick, 1984) revealed that the primary metabolic pathway is the hydroxylation of the C-8’ proline atom in the peptidic part. Splitting of the peptide bond between the lysergic acid and the tripeptide moiety was also detected. In addition, the metabolism of ergot-related drugs has been studied with some semisynthetic derivatives: lisuride (Toda and Oshino, 1981), pergolide (Rubin et al., 1981; Bowsher et al., 1992), methysergide (Bredberg and Paalzov, 1990), proterguride (Krause et al., 1993), nicergoline (Kohlenberg-Müller et al., 1991; Banno et al., 1991b; Sioufy et al., 1992), CQA 206–291 (Ball et al., 1992), cabergoline (Rains et al., 1995), sergolexole (Brooks et al., 1997), and LSD (Nelson and Foltz, 1992a, b; White et al., 1997). These studies revealed that the ergolene (ergoline) moiety can also be modified at several positions including N(1)-dealkylation (methysergide, nicergoline), N(6)-dealkylation (lisuride, cabergoline, pergolide, LSD), N-dealkylation in the urea or sulfonamide moiety (lisuride, CQA 206–291, proterguride, cabergoline), hydroxylation at the C-13 or C-15 ergolene atoms (lisuride, LSD), and formation of 2-oxo derivatives (proterguride, LSD). Glucuronides are supposed to be among the structures being excreted (Toda and Oshino, 1981; Maurer et al., 1982, 1983; Nelson and Foltz, 1992a). However, in a number of cases, only the principal metabolites were identified.

The following table lists some of the alkaloid classes, matrices, and references:

<table>
<thead>
<tr>
<th>Alkaloid class</th>
<th>Matrix</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semisynthetic drugs</td>
<td>LSD in biological fluids</td>
<td>Christie et al., 1976; Harzer, 1982; Webb et al., 1996</td>
</tr>
<tr>
<td>LSD review</td>
<td></td>
<td>Nelson and Foltz, 1992a</td>
</tr>
<tr>
<td>nicergoline impurities</td>
<td>bromokryptine in plasma</td>
<td>Flieger et al., 1984b</td>
</tr>
<tr>
<td>nicergoline in plasma</td>
<td></td>
<td>Phelan et al., 1990</td>
</tr>
<tr>
<td>lisuride in plasma</td>
<td>bromokryptine in tablet formulation</td>
<td>Kohlenberg-Müller et al., 1991</td>
</tr>
<tr>
<td></td>
<td>sergolexole and LY215840</td>
<td>Wolthers et al., 1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Foda and Elkhafie, 1996</td>
</tr>
</tbody>
</table>

Table 2 (Continued)
and the structures of others remain to be elucidated (e.g., Rubin et al., 1981). Isolation of several natural ergopeptines or clavine derivatives hydroxylated at the C-8 atom (Pakhomova et al., 1996a; Cvak et al., 1997) indicates that, e.g., also metabolites of this type can be expected. This simple list of sites of possible metabolic attack clearly illustrates that the metabolism of EA is still far from being completely understood, offering thus opportunities for future research (see also the chapter 9 on EA biotransformation in this book).

It is worth mentioning that in modern history, practically since 1993, the majority of new EA was isolated by RP-HPLC (Flieger et al., 1993; Cvak et al., 1994; Szántay et al., 1994; Cvak et al., 1997). Analysis of EA in various agricultural products dominates among other topics (Scott and Lawrence, 1980, 1982; Rottinghaus et al., 1991; Hill et al., 1993; Shelby and Flieger, 1997). By the end of the eighties, the basic development of HPLC methods was practically finished, and individual methods no longer appear in the literature without some special use or without coupling to some identification technique (LC/MS).

In the shadow of the use of C-8 or C-18 columns, some interesting methods based on other chromatographic sorbents also have been described. Even if these methods are not used routinely, they have an undeniable importance in EA research. Some disadvantages of silica gel could be at least partly overcome also by its modification with aminopropylsilane (NH₂-columns). Mixtures of chloroform, dichloromethane or diethyl ether with aliphatic alcohols (methanol, ethanol or 2-propanol) are typically used as eluents (Wurst et al., 1978, 1979; Flieger et al., 1981, 1982, 1984a, b). The same column type was also used for the supercritical fluid chromatographic (SFC) analysis of some clavine alkaloids using carbon dioxide-methanol (9:1, v/v) as an eluent (Berry et al., 1986). The merits inherent in the use of amino-modified columns are several. Firstly, as expected, the elution of EA corresponds to “normal phase” chromatography and is thus complementary to “reversed-phase” chromatography on C-18 columns. Secondly, the method very sensitively responds to even subtle stereochemical changes in the structure, e.g., epimerization at proline or other part of the peptide moiety, conversion to -inines, etc., and makes it possible, e.g., to resolve α-ergopeptines (containing Leu) and β-ergopeptines (Ile). This approach provided an almost complete resolution of the clavine and ergopeptine series (Wurst et al., 1978, 1979) and also contributed to the isolation of some new ergopeptines (Flieger et al., 1981, 1984a).

10.5. ELECTROANALYTICAL METHODS

In contrast to various chromatographic techniques, much less attention has been paid to the development of electroanalytical methods for the determination of EA. However, these alkaloids possess several features which could be used to develop such a method. Titration of EA in nonaqueous solvents with
potentiometrical endpoint determination (glass/calomel electrode system) belongs to the oldest, but in various pharmacopeias still surviving, techniques (Gyenes and Bayer, 1961). Although it is not an electroanalytical procedure, the separation of EA by counter-current distribution is also based on the difference of their dissociation constants (Galeffi and Miranda delle Monache, 1974).

Electrophoretic analysis of EA has been tested on various thin layers (Agurell, 1965; Kornhauser and Perpar, 1965, 1967). For example, silica gel G plates sprayed with an acetic acid-pyridine buffer (pH 5.6) were used for the separation at 1500 volts (Agurell, 1965). With the exception of ergopeptines, which showed some tailing tendency, EA appeared as distinct spots and the resolution was comparable to that obtained by TLC. Although the basic principle of separation by electrophoresis is different from TLC, which might have some value for the separation of complex mixtures, the method has not found its followers most probably due to the fact that the same information can be obtained more simply by a combination of several TLC methods.

Much later, the same principle was used to separate ergot alkaloids by capillary electrophoresis. Fanali et al. (1992) have demonstrated the resolution of some EA enantiomers and epimers using capillary electrophoresis with the background electrolyte supplemented with $\gamma$-cyclodextrin. Although this method is very interesting, it should be noted that the analytical problem is slightly artificial, since ergot alkaloid enantiomers are encountered neither in nature nor in synthetic mixtures, due to the fact that they are synthesized from different precursors. The use of EA derivatives themselves as chiral selectors in capillary electrophoresis is much better idea in terms of practical application. These compounds were developed as a simple modification of some known EA-based drugs by L. Cvak and applied as chiral selectors both in capillary electrophoresis (Ingelse et al., 1996a, b) and HPLC (Sinibaldi et al., 1994; Padiglioni et al., 1996; Messina et al., 1996). The same HPLC column can also be used for the resolution of some semisynthetic ergot derivatives themselves (Flieger et al., 1994).

Analyses of real samples by capillary electrophoresis were demonstrated by Ma et al. (1993). They developed a high performance capillary electrophoresis method for the determination of ergovaline in the seeds of Festuca arundinacea (tall fescue) infected with the endophytic fungus Acremonium coenophialum. Micrograms of ergovaline per kilogram of seeds can be detected and quantified. Capillary electrophoresis has also been successfully applied for the screening and quantitation of LSD in seized illicit substances (Walker et al., 1996). Only recently, the potential advantages of capillary electrophoresis have been demonstrated on the simultaneous determination of caffeine and ergotamine in pharmaceutical dosage forms (Aboul-Enein and Bakr, 1997).

Ergot alkaloids are easily oxidized in the indole moiety by a two-electron transfer process affording a deep purple highly conjugated dimer as the principal product (Dankházi et al., 1993). This feature can be used, e.g., for the electrochemical detection of EA after their HPLC separation (Pianezzola et al.,
Based on this principle, several differential pulse voltametric methods have been developed as well aimed at the analyses of a single compound (ergotamine, ergometrine, ergocristine) in a drug formulation without any preceding separation from the excipients (Belal and Anderson, 1986; Wang and Ozsoz, 1990; Inczeffy et al., 1993). The presence of bromonicotinic acid in nicergoline, a semisynthetic EA derivative, made it also possible to develop a polarographic method for its determination based on its electrochemical reduction (Sturm et al., 1992).

10.6. IMMUNOASSAYS

The main advantages of immunoassays are the possibility of analyzing very complex mixtures such as plasma and other biological fluids without extensive purification and, in comparison with chromatographic techniques, usually also with much higher sensitivity. Accordingly, the majority of immunoassays have been developed for studies of the pharmacokinetic profiles and bioequivalence studies of various dosage forms (Table 3). It is noteworthy that these methods were sometimes published separately from the subsequent pharmacokinetic studies. Thus, in order to obtain an additional information about the method performance, coefficients of variation, detection limits and usual concentrations of EA, it is advisable to read reports of these studies, e.g., for dihydroergotoxines (Kleimola et al., 1977), dihydroergopeptines and bromokryptine (Kanto, 1983), α-dihydroergokryptine (Grognet et al., 1991), lisuride (Krause et al., 1991 a), dihydroergotamine (Wyss et al., 1991; Lau et al., 1994; Humbert et al., 1996), dihydroergocristine (Grognet et al., 1992; Coppi et al., 1992), bromokryptine (Rabey et al., 1990), or cabergoline (Persiani et al., 1994; Andreoti et al., 1995). However, it should be noted that once the therapeutic dose has been established, the routine monitoring of the EA plasma levels is not necessary in clinical practice.

The possibility of a routine analysis of a large number of samples is very useful. However, the limiting factor of all immunological methods is the cross reactivity with molecules having similar epitopes. Such structurally related molecules that might be naturally present in the matrix (nonspecific binding) could originate from related structures (e.g., from related alkaloids in the fermentation broths) and in most cases also from some metabolites of the parent compound. In contrast to the chromatographic methods, which provide information on the concentration of any resolved compound or metabolite, depending on the specificity of the antibody cumulative value or specific value is the result of all immunological methods. This value always represents the sum of concentrations of all recognized compounds multiplied by their relative response factors. Accordingly, the overestimation of immunoassays with respect to a target analyte ranges typically from a few rel. % to several multiples and is strongly dependent on the antibody type. With regard to the fact that some
metabolites of EA-derivatives still retain their pharmacological activity, e.g., the metabolites of ergotamine (Tfelt-Hansen and Johnson, 1993), dihydroergotamine (Aeling, 1984; Müller-Schweinitzer, 1984), and/or pergolide (Clemens et al., 1993), the development of a specific assay for the determination of the parent compound combined with the determination of the sum of its concentration together with its metabolites by a polyclonal antibody has been
used in recent studies and is also recommended for the evaluation of bioequivalence of different dosage forms and for the comparison of various routes of administration (Lau \textit{et al.}, 1994; Ezan \textit{et al.}, 1996; Humbert \textit{et al.}, 1996).

The key step determining the specificity of an antibody is the manner of alkaloid coupling to the carrier protein used for immunization. The part of the molecule that is most distal to the site of conjugation to the carrier is that one recognized by an antibody. EA offer several possibilities of conjugation. Mannich condensation, which was originally used for conjugation of lysergic acid amide to human serum albumin (HSA) through the nitrogen atom of the indole group (Taunton-Rigby \textit{et al.}, 1973), was later used also by other authors to conjugate, e.g., ergotamine and ergocristine to bovine serum albumin (BSA) (Arens and Zenk, 1980), \textit{N}-demethylnicergoline to BSA (Bizollon \textit{et al.}, 1982), dihydroergocornine, dihydroergokryptine, or dihydroergocristine to BSA (Collignon and Pradelles, 1984), dihydroergokryptine to egg albumin (Collignon and Pradelles, 1984), or ergotamine to BSA or egg albumin (Shelby and Kelley, 1990; Shelby, 1996). This manner of preparation affords an antibody specific for the peptide portion of the molecule. For example, if the conjugate of some member of the ergotoxine family was used for the haptenation, the cross reactivity of all members of the ergotoxine series was detected even in the case of some modification at the lysergic acid moiety (Valente \textit{et al.}, 1996). However, metabolic hydroxylation of the cyclopeptide moiety diminished the cross reactivity (Valente \textit{et al.}, 1996). There is a good evidence that both alkyl groups of the first and the second amino acid of the cyclol moiety are involved in the recognition by an antibody. Examples illustrating this fact are the absence of cross reactivity of ergovaline against ergotamine antibodies, low reactivity of ergotamine against ergotoxine antibodies, and differences within the ergotoxine series (Shelby, 1996; Valente \textit{et al.}, 1996).

Alternatively, EA having a carboxylic group can be simply conjugated via an amide bond with the free amino group of a carrier using carbodiimides as coupling reagents. This method was used for binding the \textit{N}-6-carboxymethyl derivative of dihydroergotamine to BSA (Rosenthaler and Munzer, 1976), lysergic acid to polylysine (Lopatin and Voss, 1974; Shelby and Kelley, 1991), bromolysergic acid to BSA (Valente \textit{et al.}, 1996), lysergic acid to a hydrazide derivative of agarose (Loeffler and Hinds, 1975), and/or 2-(10\textalpha-methoxy6-methylergoline-8\textbeta-methoxy)-acetic acid to BSA (Chen \textit{et al.}, 1996). A two-step modification of this procedure consists in the esterification of a free OH group of an ergot derivative with succinic or glutaric anhydride to produce hemisuccinate or hemiglutarate esters. The free carboxyl group is subsequently conjugated to a carrier by the carbodiimide method. This technique was used to conjugate, e.g., ergometrine or lysergol to HSA (Shelby and Kelley, 1991; Hill \textit{et al.}, 1994; Hill and Agee, 1994). Such antibodies exhibit affinity for various compounds with an intact ergoline skeleton (i.e., both ergopeptines and clavines), but they have little affinity for compounds modified in this part.
(bromination, hydrogenation). Hence, immunoassay based on these antibodies can be used for monitoring the total content of alkaloids in fermentation broths or in agricultural products. It is worth mentioning that also EA-producing endophytic fungi can be directly detected by an immunoassay (Miles et al., 1996).

Purified specific antibodies have also been used to develop immunoaffinity chromatographic methods for on-line purification of EA from biological samples. Extracts from urine, bile, serum, and tissue are directly injected on the column with the covalently bound antibodies with little or no sample preparation (Loeffler and Hinds, 1975; Francis and Craston, 1996). The specific binding of an analyte to an antibody renders it possible to remove most of the interfering substances and permits its trace enrichment so that the subsequent analysis by conventional techniques (usually GC/MS or HPLC/MS) can provide its unequivocal identification in forensic samples. An alternative to covalently bound antibody columns is the use of a system in which a small amount of an antibody is held on a column by non-covalent interaction (e.g., by using G-protein), and, after each purification procedure, washed off the column together with an analyte. Then the chromatographic column is equilibrated with the fresh portion of an antibody (Rule and Henion, 1992; Webb et al., 1996). A combination with column-switching techniques could be used which would permit automated sample extraction with an on-line mass spectral identification of drugs directly from urine.

10.7. COMBINED MASS SPECTROMETRIC TECHNIQUES

The result of one-dimensional techniques such as GC, HPLC or TLC is a peak or a spot whose elution time or position is characteristic for a given compound and the area under the curve or the peak height is directly proportional to its concentration. However, these techniques do not provide any proof of whether a given peak or spot represents actually the target compound or whether it is an interference eluting at the same position. With the more complex matrices and with an increasing number of peaks the correct assignment becomes more and more ambiguous. Determination of LSD, whose doses as low as 25 µg can cause central nervous system disturbances, is the typical example (Clark, 1989). Hence, concentration of LSD and its metabolites in plasma are likely to be very low. At this concentration level, both gas chromatography and high-performance liquid chromatography with fluorescence detection exhibit some interferences that might prevent conclusive drug identification. Furthermore, the analyte volatility, its thermal instability and tendency to incur adsorptive losses during gas chromatographic analysis, all contribute to the difficulty of developing a method for unambiguous confirmation of LSD in body fluids (Lim et al., 1988). Similarly, since the therapeutic dose of nearly all EA is very low, also a number of other analytical methods are operating on the edge of the limit of detection.
Whereas the UV-absorbance or fluorescence are features common for many compounds, monitoring of a particular molecular ion or a characteristic fragment provides much more selective detection. This allows a sensitive determination of a component in a rather complex matrix without previous separation; a combination of the retention time with the molecular mass information then provides a strong evidence of the target structure. Tandem mass spectrometry techniques (MS/MS) can distinguish nearly unambiguously between individual structures even in the case of several isobaric compounds (having the same molecular weight) (Halada et al., 1997). The MS/MS instrumentation itself can be considered as a separation technique. This approach of MS/MS is to utilize one stage of the mass separation to isolate the compound of interest from the matrix and a second stage of the mass separation for analysis. Thus, without any preceding separation, EA can be identified directly in complex mixtures (Plattner et al., 1983; Yates et al., 1985; Belesky et al., 1988). Contamination of dairy food by ergot sclerotia can be indirectly proved using GC/MS trace analysis of ricinoleate, a special and prominent feature of oil-rich ergot tissue (Mantle, 1996). Selective ion monitoring makes it possible to reach very low limits of detection that are particularly useful in pharmacokinetic studies (Haering et al., 1985; Sanders et al., 1986; Häring et al., 1988). It should be mentioned that mass spectrometry together with NMR spectroscopy and X-ray crystallography belong also to the most powerful identification techniques.

As the GC/MS interface is relatively simple, gas chromatographic/mass spectrometric methods were the first coupled techniques used for the analyses of LSD and mass fragmentographic studies of ergopeptines. The combination of liquid chromatography/mass spectrometry (LC/MS) has been introduced in the seventies. The major drawback of this method is certainly the flow rate typically used with the high performance liquid chromatographic columns interfering with the high vacuum necessary for the performance of mass spectrometers. Consequently, the routine application of LC/MS has been waiting about 20 years for the development of a suitable LC/MS interface (Hopfgartner et al., 1993). At present, several such devices are available, and in a few cases they have already been used for EA analyses (Table 4). However, it should be noted that regardless of the development of hyphenated methods, the importance of pre-purification steps does not decrease, and in some cases the chromatographic or mass spectrometric analysis represents only a short final step.

10.8. STANDARD DEFINITION AND PHASE ANALYSIS

Continuously increasing demands on the quality of pharmaceuticals tighten up also the criteria for validation of analytical methods. No wonder, therefore, that this development includes also the quality of the analytical standards. The presence of various polymorphs in drugs can dramatically influence their pharmacokinetic profile (differences in dissolution kinetics). Similarly, the presence
of a solvate in the analytical standard can significantly affect the slope of the calibration curve. Since various polymorphs and solvates differ in the IR spectra, the use of an incorrect standard can also cause a misidentification (compare, e.g., Harris and Kane, 1991; with Neville et al., 1992). Differences in the X-ray

<table>
<thead>
<tr>
<th>Method</th>
<th>Topic</th>
<th>Reference</th>
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<tbody>
<tr>
<td>GC/MS (EI, CI, CID)</td>
<td>LisD</td>
<td>Nichols et al., 1983;</td>
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<tr>
<td></td>
<td></td>
<td>Paul et al., 1987, 1990;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Francom et al., 1988;</td>
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<td></td>
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<td>Lim et al., 1988;</td>
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<td></td>
<td></td>
<td>Papac and Foltz, 1990;</td>
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<td></td>
<td></td>
<td>Nelson and Foltz, 1992a,b;</td>
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<td></td>
<td></td>
<td>Bukowski and Eaton, 1993;</td>
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<td></td>
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<td>Nakahara et al., 1996</td>
</tr>
<tr>
<td>GC/MF/MS (CI, EI)</td>
<td>ergopeptines</td>
<td>Van Mansvelt et al., 1978;</td>
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<td></td>
<td></td>
<td>Feng et al., 1992;</td>
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<td></td>
<td></td>
<td>Uboh et al., 1995</td>
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<tr>
<td></td>
<td>dihydroergopeptines</td>
<td>Plomp et al., 1978</td>
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<td></td>
<td>bromokryptine</td>
<td>Larsen et al., 1979</td>
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<tr>
<td>SFC/EI/MS</td>
<td><em>C. purpurea</em> extracts</td>
<td>Berry et al., 1986</td>
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<tr>
<td>HPLC/EI/CI/MS</td>
<td><em>C. purpurea</em> extracts</td>
<td>Ecker et al., 1982</td>
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<td>HPLC/CI/MS</td>
<td>bromokryptine</td>
<td>Schellenberg et al., 1987</td>
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<td>HPLC/APCI/MS</td>
<td>nicergoline metabolites</td>
<td>Banno et al., 1991b;</td>
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<td></td>
<td>Banno and Horimoto, 1991</td>
</tr>
<tr>
<td>HPLC/IS, ESI/MS</td>
<td>LisD</td>
<td>Duffin et al., 1992;</td>
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<td>Hopfgartner et al., 1993;</td>
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<td>Webb et al., 1996;</td>
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<tr>
<td></td>
<td></td>
<td>Cai and Henion, 1996b;</td>
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<tr>
<td></td>
<td></td>
<td>White et al., 1997</td>
</tr>
<tr>
<td>HPLC/FAB/MS</td>
<td>ergopeptines, tall fescue</td>
<td>Miles et al., 1996</td>
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<tr>
<td>HPLC/ESI/MS</td>
<td></td>
<td>Shelby et al., 1997</td>
</tr>
<tr>
<td>Capillary electroforesis</td>
<td>LSD metabolism</td>
<td>Cai and Henion 1996a</td>
</tr>
<tr>
<td>Direct MS/MS</td>
<td><em>C. purpurea</em> extracts</td>
<td>Plattner et al., 1983</td>
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<td></td>
<td>ergopeptines, tall fescue</td>
<td>Yates et al., 1985</td>
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<td></td>
<td>ergotamine in plasma</td>
<td>Haring et al., 1985</td>
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<tr>
<td></td>
<td>bromokryptine, plasma</td>
<td>Haring et al., 1988</td>
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<tr>
<td></td>
<td>ergovaline, tall fescue</td>
<td>Belesky et al., 1988</td>
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<tr>
<td>TLC/SIMS</td>
<td>nicergoline</td>
<td>Banno et al., 1991a</td>
</tr>
</tbody>
</table>

*GC-gas chromatography, HPLC-high performance liquid chromatography, SFC-supercritical fluid chromatography, TLC-thin layer chromatography, MS-mass spectrometry, MF-mass fragmentography, CI-chemical ionization, EI-electron ionization, ECI-electron capture ionization, IS-ionspray, FAB-fast atom bombardment, ESI-electrospray, SIMS-secondary ion mass spectrometry.
powder diffractograms enable one to develop methods for the phase analysis of the mixtures of various crystalline forms occurring either in the bulk substances or originating from the production of pills. For example, such method was developed for the analyses of mixtures of “low” and “high” melting nicergoline polymorphic forms (Hušák et al., 1994b).

The first precedent example of inconsistence of two standards of LSD was published by Neville et al. (1992). However, according to our experience more than 50% of various EA derivatives examined within the last few years exhibit two or even more crystalline forms. In some cases, the solvates are so unstable that they decompose instantaneously upon isolation from the mother liquor. However, some solvates are considerably stable even under reduced pressure. In order to provide some data about the occurrence of polymorphism among ergot alkaloid derivatives, some examples are summarized in Table 5.

<table>
<thead>
<tr>
<th>Ergot derivative</th>
<th>Crystalline form</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Ergometrine</td>
<td>several forms, solvates?</td>
<td>Grant and Smith, 1936;</td>
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<td></td>
<td></td>
<td>Stoll and Hofmann, 1943a;</td>
</tr>
<tr>
<td>LSD o-iodobenzoate</td>
<td>monohydrate</td>
<td>Hušák et al., 1998</td>
</tr>
<tr>
<td>Ergopeptide bases</td>
<td>two forms, one hydrate?</td>
<td>Stoll and Hofmann, 1943b</td>
</tr>
<tr>
<td>Ergocristine</td>
<td>acetone solvate</td>
<td>Pakhomova et al., 1997</td>
</tr>
<tr>
<td>Ergogaline</td>
<td>monohydrate</td>
<td>Cvak et al., 1994</td>
</tr>
<tr>
<td>Ergotamine tartrate</td>
<td>methanol and ethanol solvates</td>
<td>Pakhomova et al., 1995, 1996b</td>
</tr>
<tr>
<td>Hydroxyergotamine</td>
<td>methanol solvate</td>
<td>Pakhomova et al., 1996a</td>
</tr>
<tr>
<td>8z-Hydroxy-z-ergokryptine</td>
<td>methanol solvate</td>
<td>Cvak et al., 1997</td>
</tr>
<tr>
<td>Dihydroergocristine</td>
<td>bis(dioxane) solvate</td>
<td>Čejka et al., 1997a</td>
</tr>
<tr>
<td>Dihydroergotamine</td>
<td>monohydrate</td>
<td>Čejka et al., 1979</td>
</tr>
<tr>
<td>mesylate</td>
<td></td>
<td>Hebert, 1979</td>
</tr>
<tr>
<td>Dihydroergocristine</td>
<td>monohydrate</td>
<td>Čejka et al., 1995</td>
</tr>
<tr>
<td>mesylate</td>
<td></td>
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<tr>
<td>Dihydroergotamine</td>
<td>monohydrate</td>
<td>Čejka et al., 1997b</td>
</tr>
<tr>
<td>mesylate</td>
<td></td>
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</tr>
<tr>
<td>Dihydro-β-ergokryptine</td>
<td>monohydrate</td>
<td></td>
</tr>
<tr>
<td>mesylate</td>
<td></td>
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<tr>
<td>Bromokryptine</td>
<td>isopropanol solvate</td>
<td>Camerman et al., 1979</td>
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<tr>
<td>methanesulfonate</td>
<td>unsolvated</td>
<td></td>
</tr>
<tr>
<td>Nicergoline</td>
<td>two unsolvated forms</td>
<td>Fabregas and Beneyto, 1981; Hušák et al.,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1994a, b</td>
</tr>
<tr>
<td>Terguride</td>
<td>several crystalline forms</td>
<td>Kratochvíl et al., 1993, 1994</td>
</tr>
</tbody>
</table>
Several distinct trends in the analytical development can be observed with respect to various areas of the research and use of EA. The most important area is undoubtedly the industrial production of EA and their derivatives and the subsequent production of dosage forms. As already mentioned, the majority of official assays for EA or their impurities, as described in various pharmacopeias, are still based on spectrophotometry and thin layer chromatography. Although TLC is usually cheaper and more suitable for the routine analyses of a large number of samples, its resolving power and accuracy for trace components are limited. Accordingly, the use of some HPLC method is frequently requested as an additional specification for the determination of impurities in connection with the bulk pharmaceuticals trade. It can thus be expected that these methods will be soon implemented also in pharmacopeias. Similarly, HPLC methods, and particularly LC/MS methods, have found an inreplaceable position in the analyses of final drug forms, in studies of pharmacokinetics and metabolism of EA derivatives.

Another dynamically developing area is immunoassays. Due to the fact that these methods make it possible to analyse a large number of samples without extensive purification and without an involvement of highly qualified personnel, they have a great chance to replace some chromatographic methods still applied in pharmacokinetic studies and environmental analyses. However, an important factor underlying the development of immunoassays is the determination of cross reactivities with various metabolites. Since our knowledge in this field is very limited even in the case of the most widespread LSD (see Nelson and Foltz, 1992b), it can be expected that the necessity of validation of immunoassays will stimulate also the research of the ergot alkaloid metabolism.

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