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DETERMINATION OF DRUGS IN BIOLOGICAL SAMPLES BY THIN-LAYER CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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SUMMARY

In this work thin-layer chromatography–tandem mass spectrometry (TLC–MS–MS) allowed detection and confirmation of caffeine and nicotine in human urine and of butorphanol, betamethasone, and clenbuterol in equine urine. In most cases of trace analysis of labile compounds the drugs could not be identified unless they were developed on a TLC plate, scraped from the plate and the TLC scrape eluted with a suitable organic solvent prior to MS–MS. Usually a sample prepared in this way still had several components in it, but was sufficiently cleaned up to allow collision-induced dissociation (CID) experiments to unequivocally identify the drug. In contrast, trace levels of labile drugs could not be identified by CID experiments either directly from the raw urine extracts or by thermally desorbing them from the TLC scrape.

INTRODUCTION

Tandem mass spectrometry (MS–MS) has been shown to be a highly specific and sensitive technique in targeted compound analysis[1–3]. The ability to analyze relatively complex samples for trace species offers real savings in both manpower and time over more conventional techniques of gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), and liquid chromatography–mass spectrometry (LC–MS) Examples have been published showing that direct probe analysis with an MS–MS instrument could allow many samples to be rapidly screened for the presence of specific compounds or compound classes with confirmation of the few positive samples by GC–MS or GC–MS–MS[2,3] There are other situations, however, in which it would be more efficient to screen the high volume of samples with an inexpensive technique and use the high specificity of MS–MS to provide confirmation of those samples believed to contain drug related substances In a previous paper we reported on the use of LC–MS–MS for the detection of administered drugs in equine plasma and urine extracts[4]. This earlier work demonstrated that chromatographic separation and on-line MS–MS analysis could provide clean collision-induced-dis-

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sociation (CID) mass spectra "unequivocally" identifying the compound in question, even in the presence of high levels of co-eluting material which might otherwise constitute an interference. We are now investigating the use of MS–MS for the confirmation of drugs in plasma and urine samples which have been screened by thin-layer chromatography (TLC). The goal of the work is to explore whether this combination can provide a rapid assay capable of detecting and identifying the presence of administered drugs in urine and blood with a high degree of confidence. This is of current interest to drug-testing laboratories as well as the clinical chemistry community.

Various embodiments of the combination of TLC and MS–MS can be envisaged In all cases, it is probably most efficient to use TLC alone to screen all of the samples (raw extracts), and proceed with MS–MS analysis only if a foreign substance is observed. It is desirable, once a positive is observed by TLC, to perform the analysis as quickly and as simply as possible. This might be done by introducing the sample into the ion source directly at any of several stages of cleanup ranging from the raw urine to the eluted TLC scrape. While the direct analysis of raw urine would be attractive from the point of view of simplicity, its success is unlikely in most situations due to low solute levels, the presence of the compound in conjugated form and high levels of other endogenous material The remaining options available are direct analysis of raw urine extract, direct thermal desorption of sample from the TLC silica, or elution of the TLC spot with an organic solvent followed by direct analysis of the extract. In each case the intact molecules must be vaporized and ionized, and the MS–MS must provide clean CID spectra of the foreign substances in order to allow identification. Each type of sample analysis has advantages In this paper results are presented from a study in which a systematic comparison of each method was made for four different administered drugs Samples were screened by TLC and introduced at each stage of work-up by direct insertion probe into an atmospheric pressure ionization (API) source coupled to a triple quadrupole MS–MS instrument.

EXPERIMENTAL

Materials

Authentic standards of each drug were obtained from commercially available sources. Caffeine was purchased from Eastman (Rochester, NY, U.S.A.) and used without further purification Butorphanol was isolated from injectable Stadol tartrate (Bristol Labs., Syracuse, NY, U S.A.) by adjusting the pH to 10 and extraction (twice) with light petroleum. Clenbuterol was isolated from NAB 365-CL (Boehringer Ingelheim, Ridgefield, CT, U.S.A.) by adjusting the pH to 12 and extraction twice with light petroleum (b.p. 30–60°C). Preparative TLC using silica gel 60 G_{254} TLC plates (E. Merck, Darmstadt, G F.R.) produced a material that produced one spot. Authentic betamethasone was obtained from Steraloids (Wilton, NJ, U.S A.) and used without further purification

The TLC plates were stored in a desiccator and used without prior activation. Sample application to the TLC plates was accomplished utilizing disposable homemade glass capillary applicators which had been drawn out in a flame The applied sample was restricted to a spot with a maximum diameter of 2 mm by directing a gentle stream of warm air at the point of sample application. Development of the

TABLE I

TLC ANALYSIS OF THE DRUGS STUDIED

Solvent systems A = ethyl acetate–methanol–ammonium hydroxide (85 10 5), B = chloroform–methanol–propionic acid (72 18 10); C = ethyl acetate acetic acid (39 1), D = ethyl acetate–methanol–ammonium hydroxide (85 10 65)

Compound	*Solvent system*	*Visualization*	R_F
Caffeine	A	quench	0 62
Butorphanol	B	quench, Dragendorff	0 36
Betamethasone	C	quench	0 70
Clenbuterol	D	quench	0 71

respective drugs was conducted in a covered, pre-equilibrated glass developing tank containing a developing solvent mixture best suited for optimum chromatographic separation for the drug from interfering endogenous substances. Visualization of the components of interest was accomplished either under UV light or by an appropriate chromogenic spray reagent. Table I summarizes the pertinent details of the TLC analysis of the four drugs under investigation in this study

Preparative TLC involved the selective removal of the distinct, intact "spot" of each drug in question at its corresponding R_F value. This differs from conventional preparative TLC wherein an entire streaked band of the drug may be scraped from the R_F region corresponding to that known for the substance in question. The latter technique appears less desirable due to poorer resolution from potential overloading of the plate. In addition, excessive dissolved silica interferes when the chemical substance is eluted from the silica scrape in a subsequent step. We prefer the careful application of the biological extract at the origin as concise spots[5] which are scraped as a spot after development.

Spot removal is accomplished with a glass micro preparative TLC probe fabricated by drawing out the large diameter end of a 15-cm Pasteur pipette so it is symmetrical about the center. Porous polypropylene plugs were cut from No. W-11 polypropylene wicks (Schleicher and Schuell, Keene, NH, U.S A.) and gently tapped into the Pasteur pipette constriction prior to drawing out the opposite end. This device was used to scrape off gently the TLC spot of interest. A vacuum created at the opposite end of this micro preparative TLC probe sucks the silica particles from the TLC plate into the central, larger diameter region of this device. When the spot has been removed from the TLC plate the micro preparative TLC probe may be charged with a suitable solvent to elute the preparatively collected organic substance from the silica for subsequent analysis This technique is particularly well suited for recovering trace levels of drugs which may be susceptible to adsorptive losses[6].

The disposition of each drug into the urine resulted from normal excretion of administered drugs. Caffeine was extracted from the urine of a human volunteer who is a moderate coffee drinker and cigarette smoker. Butorphanol was recovered from equine urine subsequent to the administration of Stadol to a standardbred horse, betamethasone was isolated from equine urine subsequent to the administration of betamethasone and clenbuterol was isolated from equine urine subsequent to the

administration of NAB-365CL. In the latter three instances control urines were collected prior to the administration of the respective drugs and analyzed in parallel with the post administration samples.

Equipment

The mass spectrometer used in this study was a TAGA® 6000 triple quadrupole mass spectrometer system equipped with an API source[7]. All samples were introduced into the API source on glass surfaces in a direct insertion probe. In the case of liquid extracts, 2-μl aliquots were carefully deposited from a syringe onto a glass surface in which a small heating coil is inbedded. The silica was introduced by placing a few of the particles in a 2-mm glass tube with a closed end, and heating this tube in the direct insertion probe using another type plug-in heating tip. The probe was situated in a stream of carrier gas (Zero Air, Specialty Gases, Toronto, Canada) which flows into the ion source at 3 l/min. After the few seconds required for the solvent to evaporate, the heating current was slowly raised until a stable ion signal from the compound was obtained. Full-scan mass spectra and CID spectra of the parent MH^+ ions were obtained [except for betamethasone, where the CID spectrum of the $(MH^+ - 60)^+$ was obtained] Each sample was cleaned from the probe by raising the current to bake the probe for a few seconds. No memory or sample carryover was observed, and each analysis required approximately 2 min.

RESULTS

The choice of the drugs studied in this work, caffeine, butorphanol, betamethasone and clenbuterol, was dictated by a combination of their relative importance in horse racing and the degree of difficulty in their detection and confirmation by conventional methods. In particular, the recovery of caffeine by preparative TLC can be very low especially at trace levels[8]. Although caffeine can be determined by GC–MS, it exhibits considerable chromatographic peak tailing and is interfered with by endogenous components Butorphanol may also be determined by GC–MS if a suitable derivative is prepared, but its low urinary levels and thermal instability causes difficulties with its GC–MS determination. The confirmation of betamethasone in equine urine is difficult because it too must be detected at low ng/ml levels and distinguished from many endogenous steroids and other interfering substances. It is not easily amenable to GC–MS at the low levels found in equine urine. Clenbuterol is a potent respiratory stimulant that is administered at approximately 200-μg doses to horses so it is present in biological fluids at extremely low levels. Although clenbuterol may be determined by GC–MS, the extensive sample cleanup necessary to provide good GC–MS results often results in unavoidable sample loss such that GC–MS detection limits are not adequate for the trace amount of material available. Thus, reduced sample handling and direct insertion of the sample into the MS should preclude some of these difficulties.

Caffeine

The API mass spectrum of authentic caffeine is shown in Fig. 1A. The protonated molecular ion at m/z 195 is the base peak in this spectrum with only minor fragment ions at lower mass This is a desirable situation for MS–MS as reported by

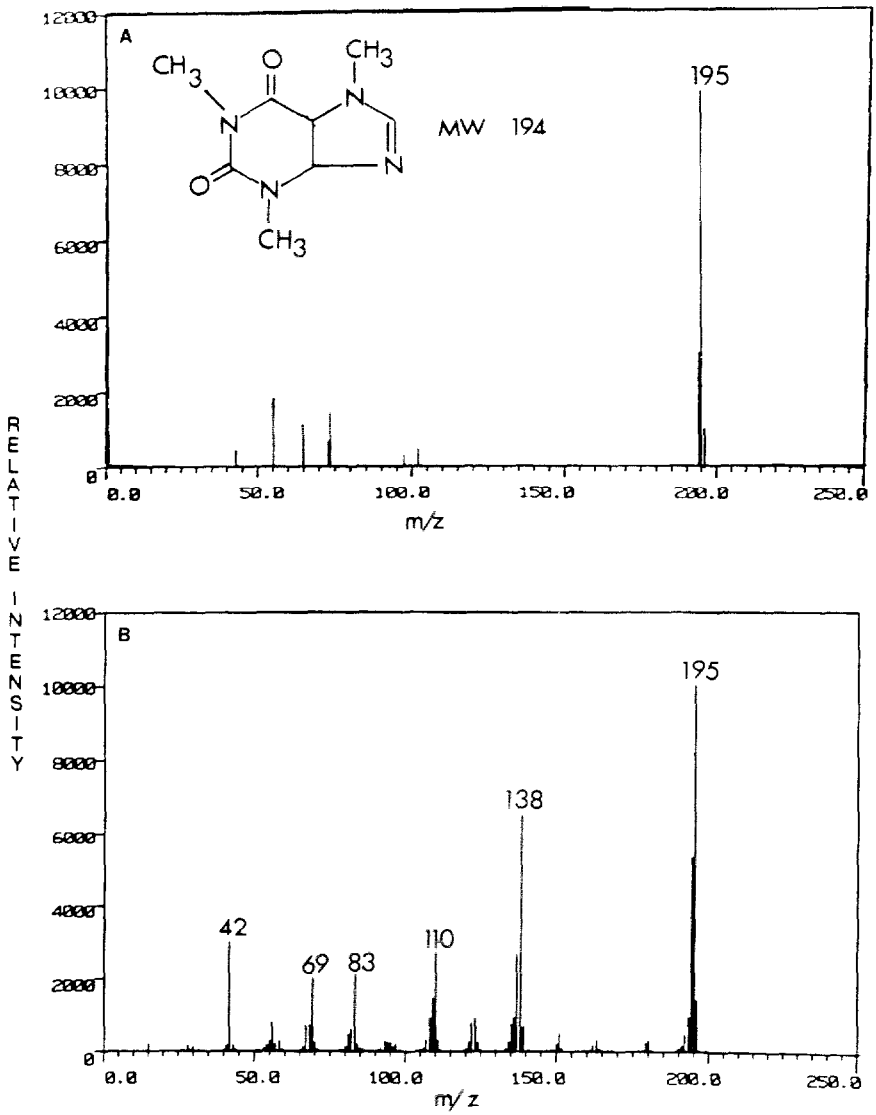

Fig. 1. (A) Full-scan direct insertion probe API mass spectrum of standard caffeine. (B) API CID mass spectrum of standard caffeine $(M + 1)^+$ ion (*m/z* 195).

others since it provides a unique ion characteristic of the molecular weight of the compound, which may be dissociated by collision with neutral gas molecules thus providing a CID mass spectrum of the $(M + 1)^+$ ion. Fig. 1B shows the CID mass spectrum for the $(M + 1)^+$ ion of caffeine using argon collision gas with a laboratory collision energy of 44 V. The daughter ion spectrum under these conditions includes abundant fragment ions at *m/z* 138, 110, 83, 69, 56 and 42 characteristic of the structure of caffeine.

The full-scan API mass spectrum of a 2-μl aliquot of a human urine extract (no

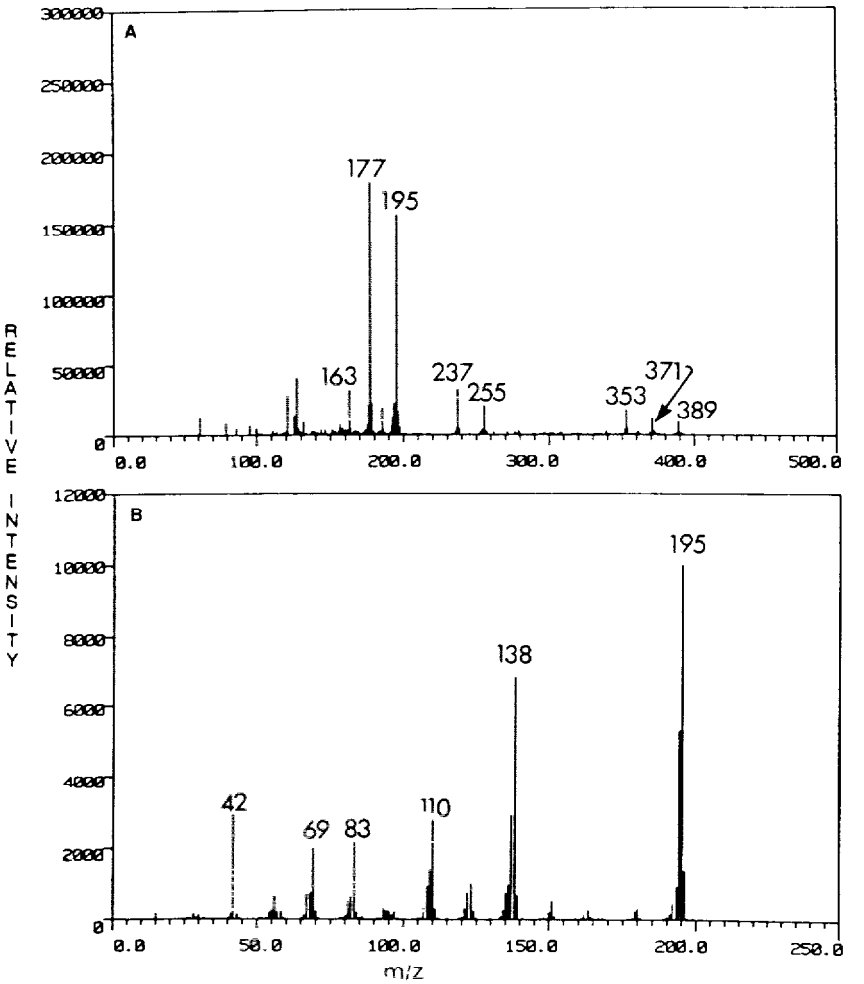

Fig 2 (A) Full-scan direct insertion probe API mass spectrum of an organic solvent extract of human urine obtained from a person who was known to drink coffee and smoke cigarettes (B) API CID mass spectrum of the human raw urine extract m/z 195 ion observed in A.

TLC clean up) is shown in Fig 2A There are several abundant ions in the molecular weight region of caffeine in addition to ions at m/z 237, 255, 353, 371 and 389 Clearly this is a "mixed" API mass spectrum, presumably indicating the protonated molecular ions of numerous organic components in this raw urine extract. However, if either TLC screening results or other information has suggested that caffeine may be in this urine extract, one can perform CID experiments on the $(M + 1)^+$ ion, or m/z 195, observed in Fig. 2A This experiment provides the CID spectrum shown in Fig. 2B which compares very well with that for standard caffeine shown in Fig 1B and verifies the identity of caffeine in the human urine extract

The effects of some sample clean up by preparative TLC are shown in Fig 3A. The components observed at higher masses in the full-scan API mass spectrum of the

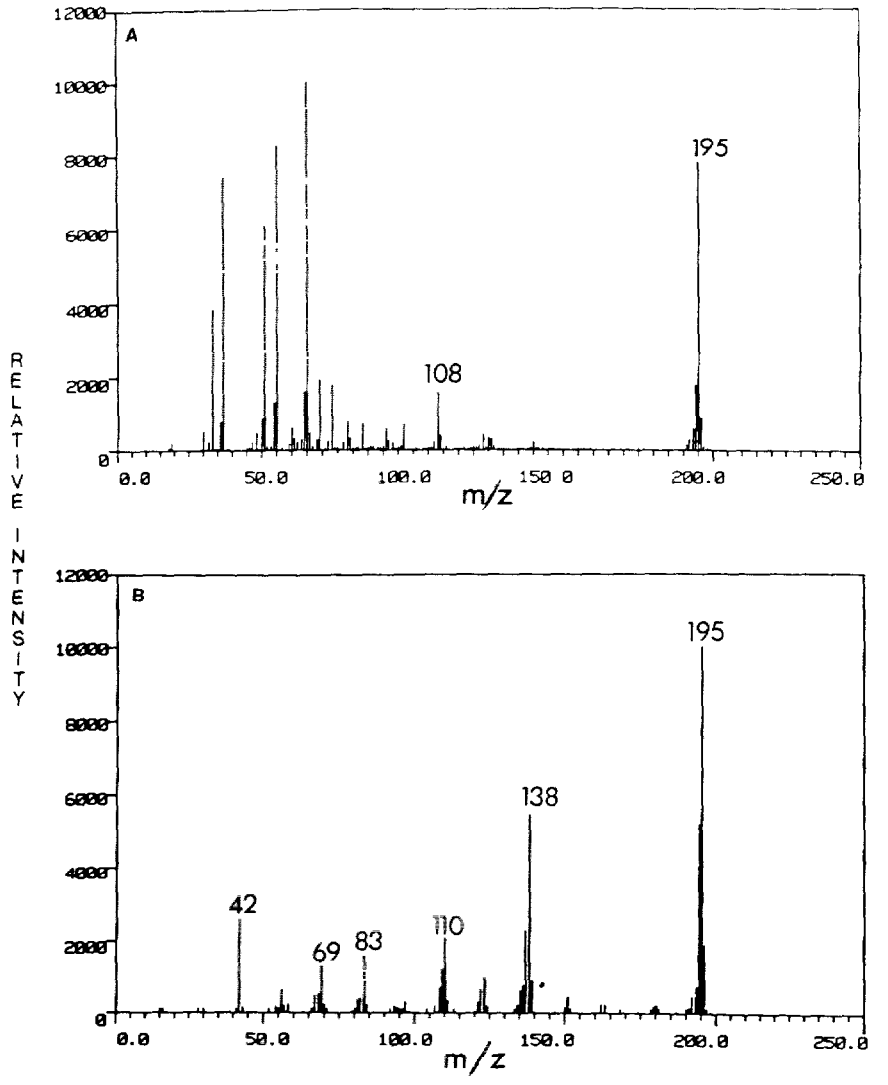

Fig 3 (A) Full-scan direct insertion probe API mass spectrum of an eluted TLC scrape obtained from the human raw urine extract described in Fig 2A (B) API CID mass spectrum of the m/z 195 ion observed in A

raw urine extract are not observed when a TLC spot of the same R_F as standard caffeine is analyzed. The full-scan API results on such an "eluted TLC scrape" are shown in Fig 3A An abundant ion at m/z 195 is observed in addition to numerous lower mass ions which are probably $(M + 1)^+$ ions of other components present in this sample This "mixed" mass spectrum is not sufficient for identifying caffeine in this instance so CID was carried out on the m/z 195 ion for caffeine whose presence had been suggested by TLC screening results The CID mass spectrum for the m/z 195 ion observed in Fig 3A is shown in Fig 3B. This mass spectrum is identical to that

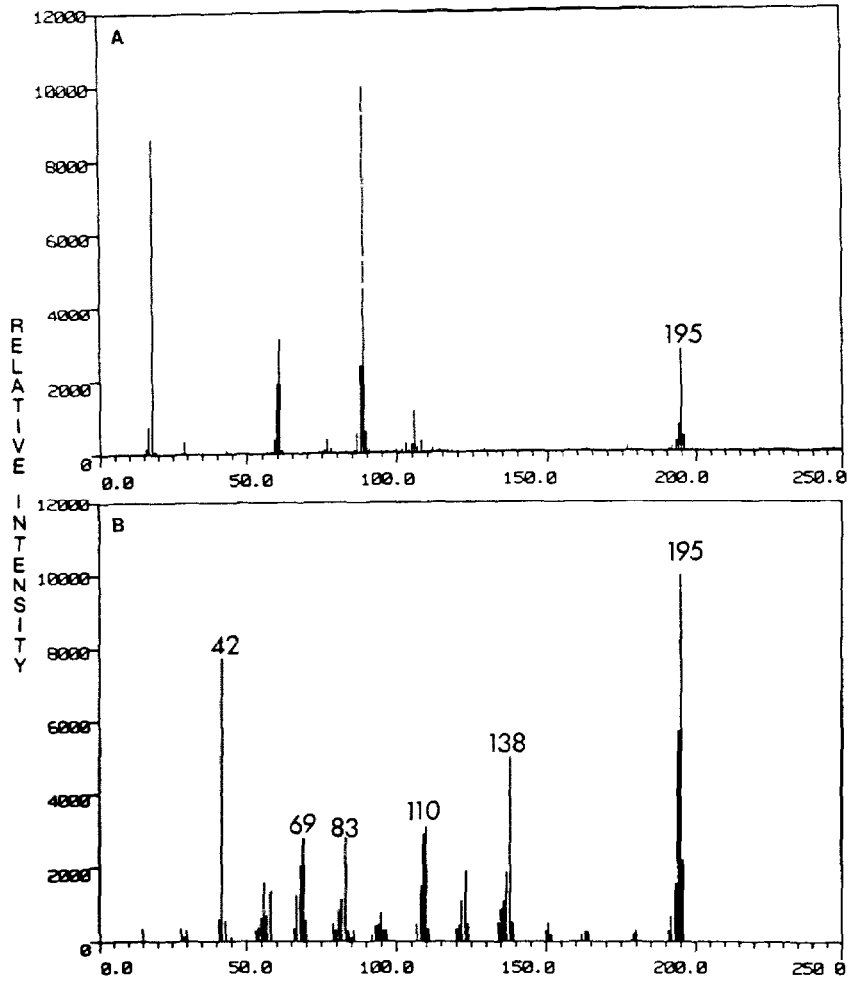

Fig. 4 (A) Full-scan direct insertion probe API mass spectrum of the TLC scraped spot not eluted with solvent. The silica particles from the scrape were placed directly on the direct insertion probe and the sample thermally desorbed (B) API CID mass spectrum of the m/z 195 ion observed in A

obtained similarly for standard caffeine (Fig 1B) and thus identifies caffeine in this eluted TLC scrape.

The direct thermal desorption of organic substances from silica isolated from a TLC scrape can be accomplished[9] This technique does not require elution of a drug, for example, from the silica surface subsequent to the preparative TLC step. The problem, however, is that certain substances that are thermally labile and present at very low levels may be thermally degraded by heating them on the silica surface. This technique, therefore, is useful only when the organic substance is present at high levels and/or is not thermally labile

Fig 4A shows the full-scan API mass spectrum of a TLC scraped spot which had been observed at an R_F identical to that of standard caffeine. The silica recovered from the surface of the TLC plate was placed directly on the probe tip, inserted into

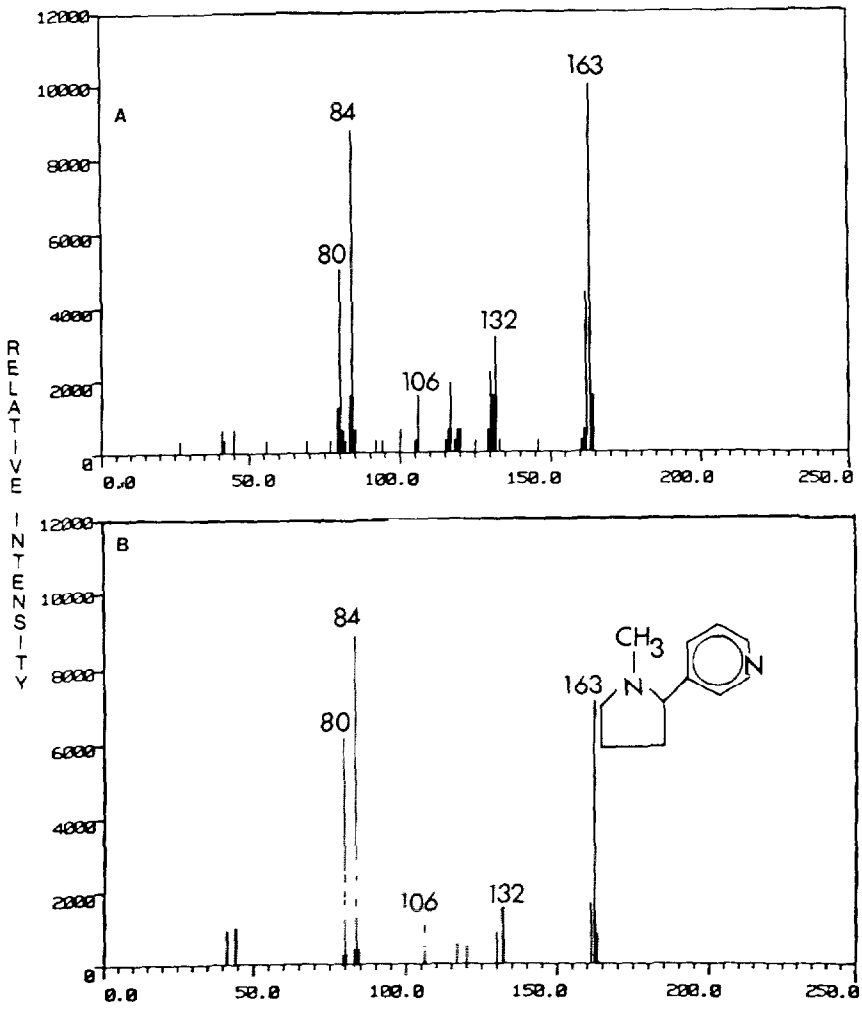

Fig 5 (A) API CID mass spectrum of the m/z 163 ion observed in Fig 2A (B) API CID mass spectrum of standard nicotine $(M + 1)^+$ ion (m/z 163)

the API source and heated to desorb any volatile substances from the surface of the silica. Fig 4A shows several abundant ions, including m/z 195. It is interesting to note, however, that there is little similarity between the mass spectra shown in Figs. 3A and 4A although they are both derived from preparative TLC scrapes of the same sample The differences observed are presumably due to the different effects caused by eluting the sample from the silica *versus* thermally desorbing the sample from the silica. The CID mass spectrum shown in Fig. 4B, however, is the same as that observed for standard caffeine and thus demonstrates that caffeine at these rather high levels can be thermally desorbed from the TLC scrape and identified by TLC–MS–MS.

The m/z 163 ion observed in the full-scan API mass spectrum of the human urine raw extract shown in Fig 2A is also of possible interest. The TLC screening re-

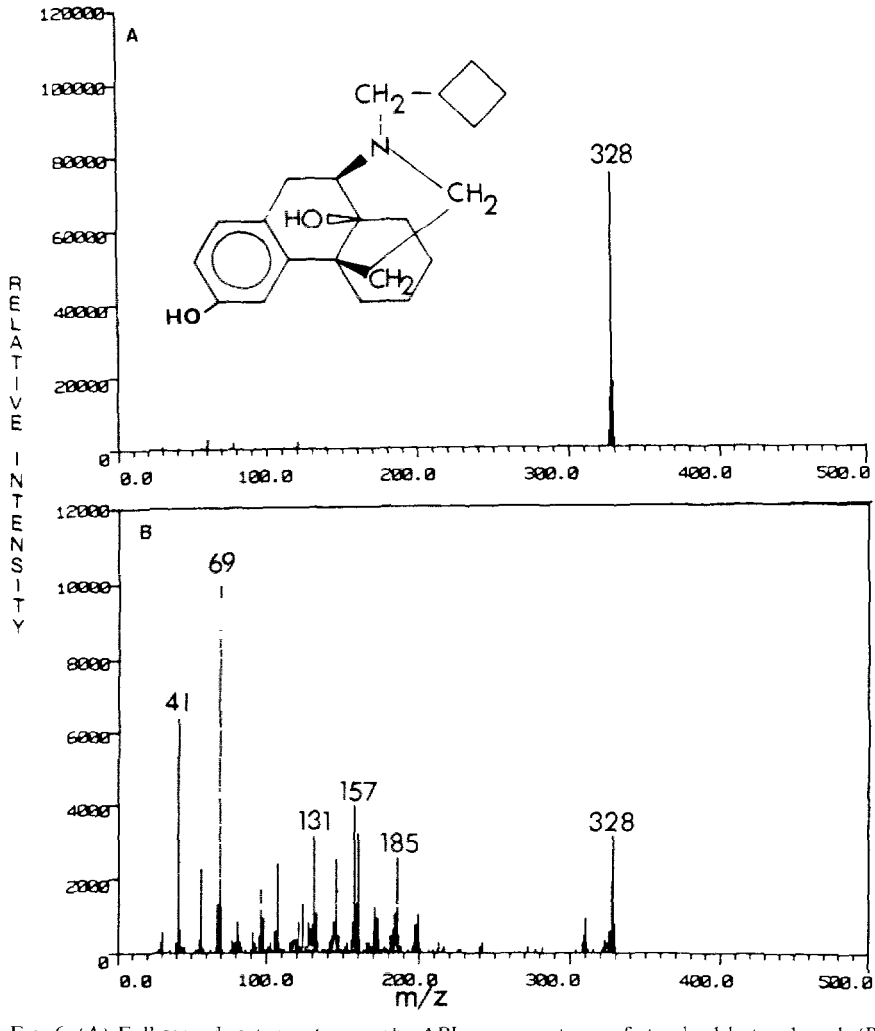

Fig 6 (A) Full-scan direct insertion probe API mass spectrum of standard butorphanol (B) API CID mass spectrum of standard butorphanol $(M + 1)^+$ ion (m/z 328)

sults described above suggested the presence of nicotine in this sample so a CID experiment was conducted on the m/z 163 ion This produced the CID mass spectrum shown in Fig 5A which compares favorably with that of a nicotine standard shown in Fig. 5B. Thus the combination of TLC screening followed by MS–MS allows the rapid identification of organic substances in a raw organic extract of urine when the concentrations of these substances are rather high.

In this human urine example caffeine and nicotine were identified in the raw extract of the urine. Although many potentially interfering substances were present. CID on the $(M + 1)^+$ ions of each component gave satisfactory results from the raw extract, the eluted TLC scrape, and the TLC scrape Clearly one does not have to bother with the latter two experiments if a high level of a targeted compound is present. However, as we shall see in later examples, one does not always enjoy this luxury

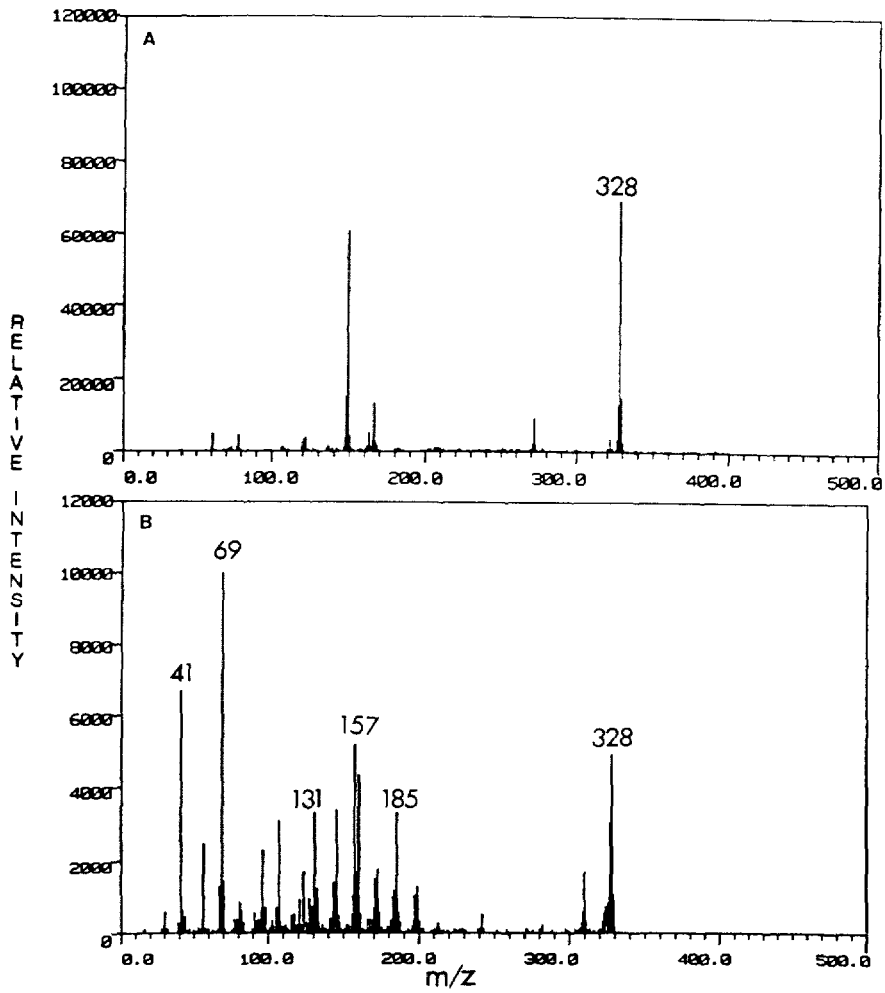

Fig 7. (A) Full-scan direct insertion probe API mass spectrum of an eluted TLC scrape obtained from an organic extract of an equine urine subsequent to the administration of butorphanol (B) API CID mass spectrum of the *m/z* 328 ion observed in A

Butorphanol

The full-scan API mass spectrum from a standard sample of the analgesic, butorphanol, shows only the protonated molecular ion at *m/z* 328 in Fig. 6A. This extremely simple mass spectrum easily verifies the molecular weight of the drug, but provides no structural specificity that is essential for "unequivocal" identification. However, the CID mass spectrum of the *m/z* 328 ion of standard butorphanol shown in Fig 6B provides a wealth of fragment ions The base peak at *m/z* 69 is accompanied by other abundant ions which include *m/z* 328, 185, 157, 131 and 41 to mention a few

In contrast to the caffeine experiments described above, neither a full-scan API nor a CID mass spectrum of a crude extract of an equine urine containing ad-

ministered butorphanol revealed any information allowing the identification of this drug in the crude urine extract This appeared to be due to intolerable matrix effects in the extremely complex equine urine extract and ng/ml levels of the drug in the urine. The complexity of equine urines compared to human urine is a well known fact and has been addressed previously[4-6]

When an eluted TLC scrape of butorphanol was analyzed by TLC–MS–MS the full-scan API mass spectrum shown in Fig. 7A was observed An abundant *m/z* 328 ion was seen in addition to several other ions that are probably the $(M + 1)^+$ ions of other organic substances eluted from the TLC spot The CID mass spectrum for the *m/z* 328 ion in Fig. 7A is shown in Fig. 7B This spectrum compares favorably with the corresponding CID spectrum for the *m/z* 328 of standard butorphanol and thus satisfactorily identifies this drug in the eluted TLC spot.

Fig. 8 shows the full-scan API mass spectrum obtained by placing the TLC scrape of silica containing butorphanol from the equine urine extract into the API source. The spot was isolated from an equine urine extract containing the administered drug. Thus the isolated drug present at about 50 ng/ml in equine urine was thermally desorbed from the silica which had been placed on the direct insertion probe. The full-scan API mass spectrum shown in Fig. 8 reveals no significant *m/z* 328 ion as was observed in Fig. 7A. Similarly, CID experiments on *m/z* 328 in this instance provided no useful CID data. Thus it appears that butorphanol cannot be thermally desorbed from a silica surface for MS analysis at these levels but satisfactory results may be obtained if the spot is first eluted with organic solvent. This is perhaps not surprising in view of the structure of this drug.

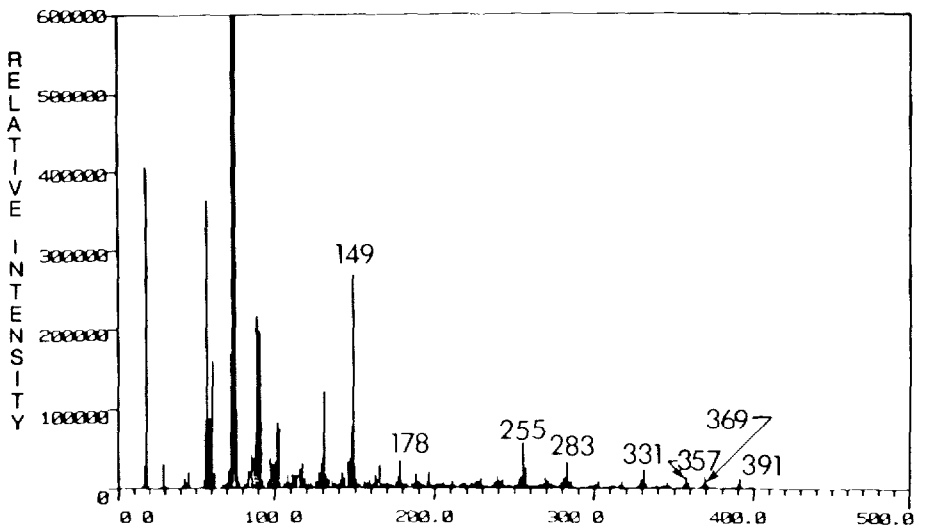

Fig 8 Full-scan direct insertion probe API mass spectrum of the TLC scraped spot obtained from an organic extract of an equine urine subsequent to the administration of butorphanol The silica particles were not eluted with solvent, but placed directly on the direct insertion probe and the volatile organics thermally desorbed from the silica

Betamethasone

The administration of the corticosteroid, betamethasone, to a horse is followed by rapid metabolism and excretion of the parent drug. Thus plasma and urinary levels are routinely quite low in spite of the fact that routine administration may be given The detection and identification of betamethasone in the presence of many other endogenous steroid related substances poses a challenging analytical problem[10].

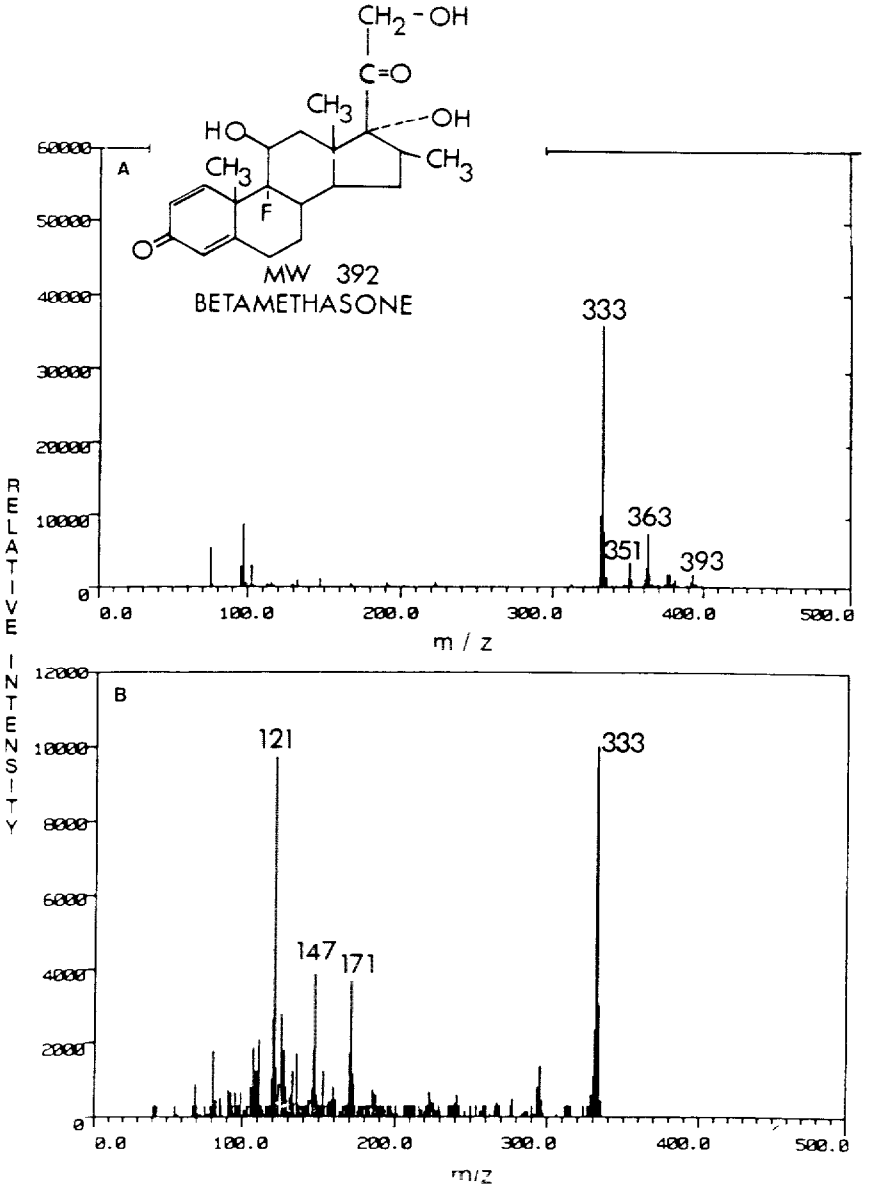

Fig. 9 (A) Full-scan direct insertion probe mass spectrum of standard betamethasone (B) API CID mass spectrum of standard betamethasone $(M + 1 - 60)^+$ ion (m/z 333)

We have developed TLC screening methods for detecting betamethasone and its metabolites in equine urine and plasma[9], but confirmation is best accomplished by micro LC–MS[10]. Although this method provides satisfactory results, considerable sample clean up and instrumental set up is required.

We have investigated the possibility of identifying betamethasone by TLC–MS–MS. Fig. 9A shows the full-scan API mass spectrum of standard betamethasone. Unfortunately, the facile loss of $C_2H_4O_2$ from the D ring[11] yields a relatively weak $(M + 1)^+$ ion. However, the structurally significant m/z 333 ion resulting from this fragmentation is the base peak and a convenient parent ion for CID experiments (Fig. 9B). This CID daughter ion mass spectrum provides sufficient specificity for identifying betamethasone in mixtures.

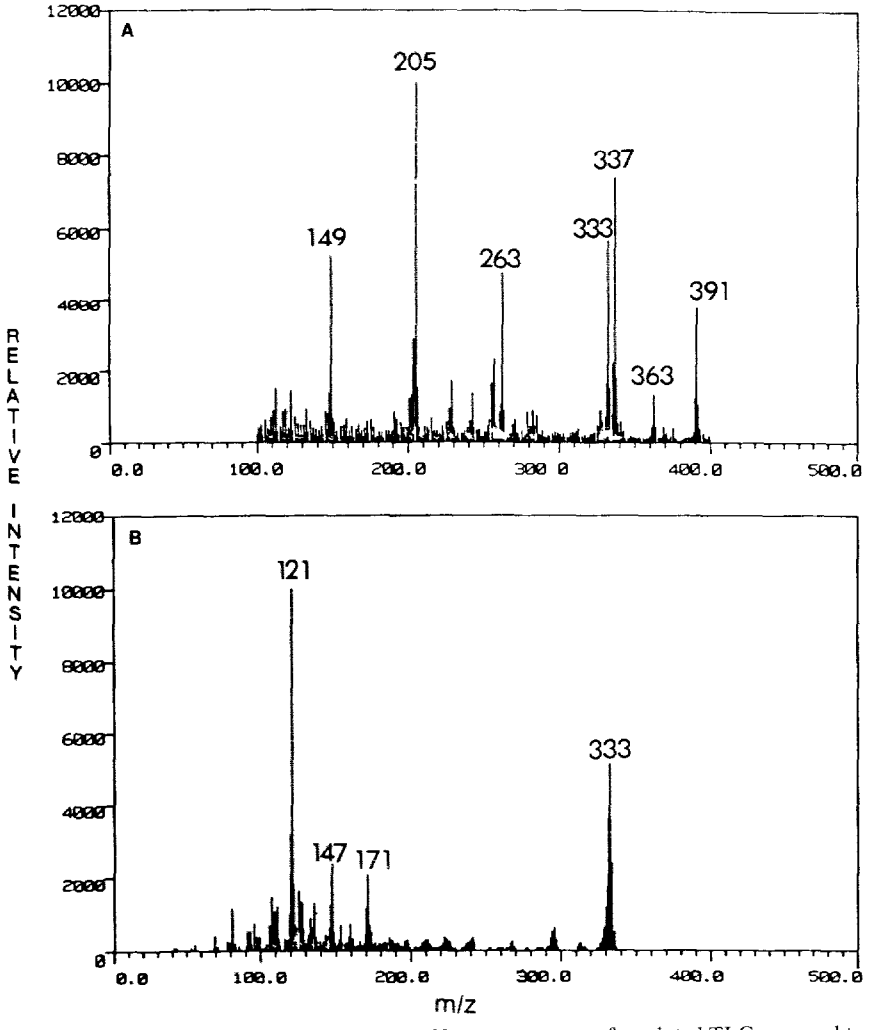

Fig 10 (A) Full-scan direct insertion probe API mass spectrum of an eluted TLC scrape obtained from an organic extract of equine urine subsequent to the administration of betamethasone. (B) API CID mass spectrum of the $m\ z$ 333 ion observed in A

The full-scan API mass spectrum shown in Fig. 10A was obtained from an eluted TLC scrape of an equine urine obtained after a betamethasone administration The eluted spot was removed from a TLC plate at an R_F that was the same as that of standard betamethasone As can be seen from Fig. 10A there are numerous ions present in addition to those expected from betamethasone However, if a CID experiment is performed on the m/z 333 ion the daughter ion spectrum shown in Fig 10B is observed. This spectrum compares favorably with the CID mass spectrum from standard betamethasone shown in Fig. 9A and thus identifies betamethasone in this eluted TLC scrape. Comparable experiments from the raw urine extract and the TLC scrape of the betamethasone urine did not provide any useful results In this case, too, the matrix effects in the raw extract and the thermal decomposition on the heated silica preclude satisfactory results from these experiments. However, the simple two-step procedure of urine extraction followed by TLC–MS–MS provides rapid, "unequivocal" identification of this important corticosteroid in equine urine.

Clenbuterol

Clenbuterol is a potent respiratory stimulant administered to horses in doses ranging from 100 to 200 μg. The rapid onset of pharmacological effects coupled with a short duration are manifested by low levels of the parent drug in the plasma and urine. The detection of clenbuterol in equine urine and its confirmation by GC–MS can be accomplished by scrupulous preparative TLC procedures and derivatization prior to GC–MS analysis on well conditioned GC columns[8]. A simplified confirmation step requiring less sample cleanup is clearly desirable due to the sample losses resulting from multistep clean up.

Fig 11A shows the full-scan API mass spectrum of standard clenbuterol The base peak at m/z 277 readily documents this drug's molecular weight of 276 with a few fragment ions characteristic of the benzylic secondary amine side chain moiety appearing at lower masses. The CID daughter ion spectrum for m/z 277 shown in Fig 11B displays fragment ions at m/z 259, 203, 168, 132 and 57.

The full-scan API mass spectrum shown in Fig 12A was obtained from an eluted TLC scrape of an equine urine extract obtained after intravenous administration of 200 μg of clenbuterol. The developed spot was removed from the TLC plate at the R_F equal to that observed for standard clenbuterol using the micro spot scraper described above. This small amount of silica was eluted with methanol to recover the organic material adsorbed to its surface. The resulting methanol solution was concentrated to about 10 μl and a 2-μl aliquot was placed on the direct insertion probe. Fig. 12A shows the full-scan API mass spectrum of this eluted TLC scrape. Although there is a relatively small m/z 277 ion observed, there are many other abundant ions present that preclude identification of clenbuterol from this mixed mass spectrum. However, if CID is performed on the m/z 277 ion, the daughter ion mass spectrum shown in Fig. 12B is obtained, which agrees well with the CID mass spectrum obtained from standard clenbuterol shown in Fig 11B. This TLC–MS–MS experiment allows the "unequivocal" identification of clenbuterol from the eluted TLC screening plate following minimal but essential sample cleanup.

Similar TLC–MS and TLC–MS–MS experiments on the raw urine extract and the TLC scrape analyzed directly on the direct insertion probe without elution by methanol failed to provide useful MS data for the identification of clenbuterol in the

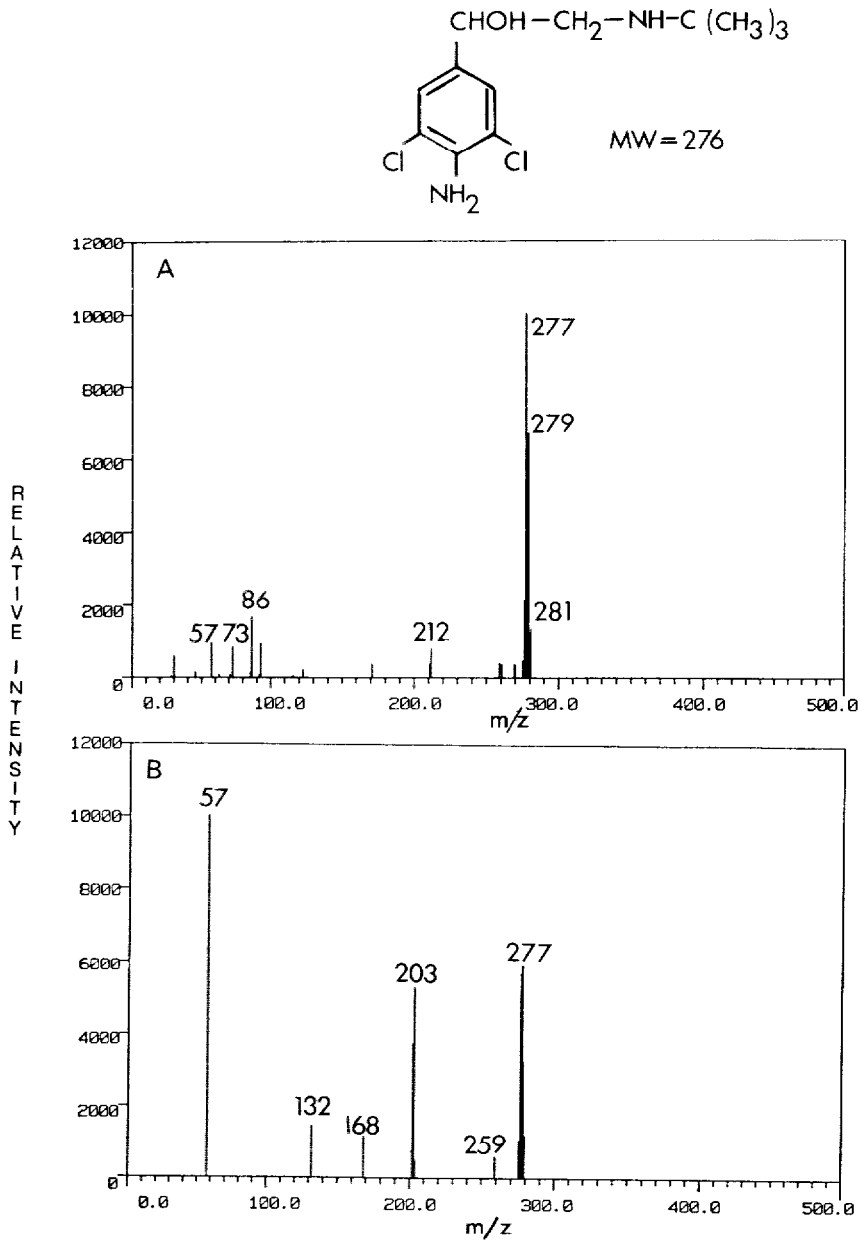

Fig. 11. (A) Full-scan direct insertion probe mass spectrum of standard clenbuterol. (B) API CID mass spectrum of standard clenbuterol $(M + 1)^+$ ion (*m/z* 277).

equine urine extract. In the former case the matrix effects resulting from the complexity of the urine extract precluded useful MS data. In the latter case thermal desorption of the labile drug from the surface of the silica particles produced no $(M + 1)^+$ ions for clenbuterol presumably due to decomposition of the drug on the silica prior

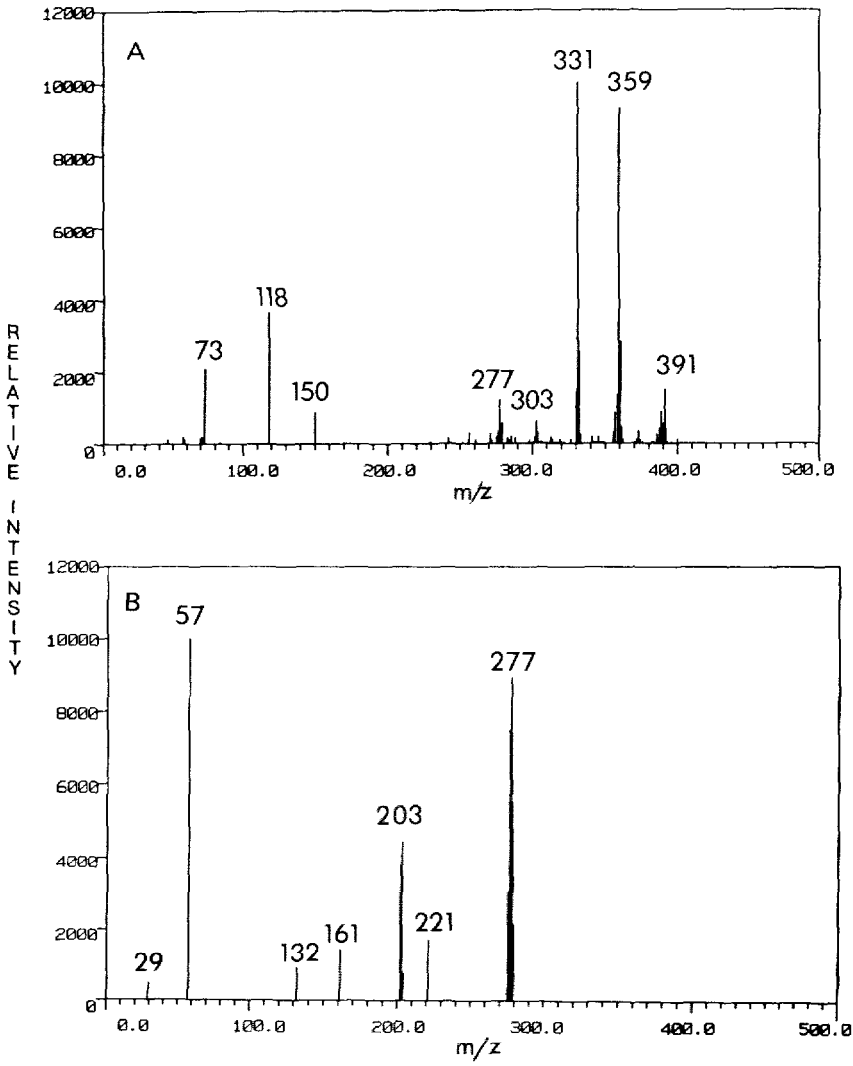

Fig 12 (A) Full-scan direct insertion probe API mass spectrum of an eluted TLC scrape obtained from an organic extract of equine urine subsequent to the administration of clenbuterol. (B) API CID mass spectrum of the *m/z* 277 ion observed in A.

to chemical ionization Thus, as was observed above for both butorphanol and betamethasone, the technique of TLC–MS–MS is well suited for rapid identification of clenbuterol after it has been isolated from the major endogenous components present in the organic extract of the equine urine by solvent elution from the TLC silica However, both matrix effects and catalytic effects appear to preclude successful identification of these drugs at trace levels by the method described in this work

DISCUSSION

The four drugs chosen for this work vary in the degree of difficulty for their identification in biological fluids. In this work caffeine and nicotine in the human urine of a chronic coffee drinker and cigarette smoker were relatively easy to detect and identify. Thus, these drugs were easily determined in the raw extract, the eluted TLC scrape and the TLC scrape itself. This was the only case in the examples cited where this was possible. We feel this was due primarily to the μg/ml concentrations of these substances It is known, for example, that caffeine is very difficult to elute from a TLC spot when it is present at low ng/ml levels[8].

The other drugs in this study enjoyed a similarity with respect to their rather low urine concentrations and thermal instability. Each of these factors contribute to analytical difficulties when it comes to their detection and identification in biological fluids. As is often the case, the more the analyst handles a sample during workup the more likely sample loss will occur with additional sample purification steps. We have endeavored. therefore, to combine the highly successful TLC screening technique with the relatively new MS–MS technique to provide high volume screening capability with unmatched high specificity mixture analysis We introduced a fast TLC sample clean up step that has been shown to be very effective This is demonstrated by the lack of success with CID of raw urine extracts and TLC scrapes of butorphanol, betamethasone and clenbuterol, but favorable results from the eluted TLC scrapes for these drugs. This viewpoint is in contrast to earlier reports of MS–MS experiments on untreated samples[1–3], but in line with a more practical concensus developing[4,12].

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