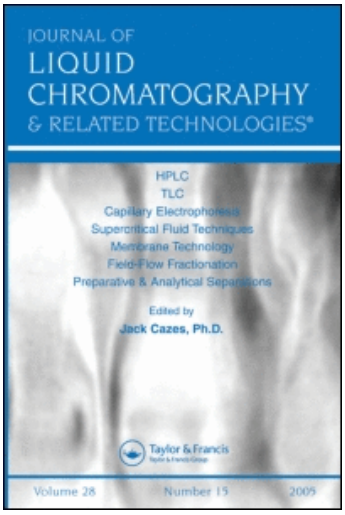


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Identification of Barbiturates Using High Performance Liquid Chromatography-Particle Beam EI/CI Mass Spectroscopy

T. W. Ryan[a]
[a] Ganes Chemicals, Inc., Pennsville, New Jersey

Online publication date: 14 May 2010

To cite this Article Ryan, T. W.(1994) 'Identification of Barbiturates Using High Performance Liquid Chromatography-Particle Beam EI/CI Mass Spectroscopy', Journal of Liquid Chromatography & Related Technologies, 17: 4, 867 — 881
To link to this Article: DOI: 10.1080/10826079408013374
URL: http://dx.doi.org/10.1080/10826079408013374

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(4), 867-881 (1994)

IDENTIFICATION OF BARBITURATES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-PARTICLE BEAM EI/CI MASS SPECTROSCOPY

TIMOTHY W. RYAN
Ganes Chemicals, Inc.
33 Industrial Park Road
Pennsville, New Jersey 08070

ABSTRACT

The procedure described uses a high performance liquid chromatograph (HPLC) coupled to a quadrupole mass spectrometer via a particle beam interface for the determination of barbiturate identity. The object was the development of a general LC-MS method capable of performing routine analysis. The use of the particle beam interface as a sample introduction technique was selected because of its relatively simple nature and the ability to adapt existing HPLC methods with only minor modification. Chemical ionization (CI) using methane as a reagent gas generated base peak molecular ions for all of the barbiturates analyzed. Electron ionization (EI) at 70 eV caused sufficient fragmentation to allow the analyst to discriminate barbiturates when identical molecular weights were encountered. In those case where highly similar EI and CI spectra are produced, chromatographic separation provided clear distinction of analyte identity. Conversely, when very different spectra are encountered, base line resolution of the analytes was not necessary for accurate identification. When nearly co-eluting spectrally similar analytes are encountered, careful evaluation of the EI+ mass spectra revealed the presence of specific mass peaks exclusive to a particular barbiturate. These mass peaks are used to confirm identity.

INTRODUCTION

Mass spectroscopy (MS) represents an ideal detection system for many disciplines. The ability of the technique to provide structural information on an analyte allows the analyst to determine identity without relying on a reference standard for comparison. This is particularly useful when analytes take the form of metabolites, degradation products, or impurities contributed by the manufacturing process. As system hardware and controlling software become more sophisticated and reliable, mass spectrometry is finding its way out of R&D laboratories into more routine environments, such as analytical and quality control labs. In view of an ever stricter regulatory climate for many aspects of pharmaceutical manufacturing, MS may soon be a required technique for such exercises as purity profiles and process validation.

A variety of techniques for the identification of barbiturates are discussed in the literature. UV photodiode array[1-3] and dual wavelength detection[4] can provide very accurate means of identification, but require a known standard and therefore are limited when an unknown compound is encountered. Although some structural deductions can be provided by the UV spectra[5], when an unknown is encountered UV photodiode array is limited to determining what the compound is *not*, and such "negative results" are *not* the goal of an analytical laboratory. A number of GC-MS methods have been documented but many[6-9] require derivatization, although there are exceptions[10,11]. All employ EI or CI (or a combination of both) despite claims that simple EI/CI spectra do not provide readily available identification of barbiturates[12].

There are many HPLC methods that rely on retention matching for peak identification[13-17]. However, the establishment of retention databases[18] for barbiturate identification can be limited by occurrences of coelution and retention wandering that may result from day to day method variations, despite the use of an internal reference standard. Coupled with an appropriate extraction method, a reliable LC-MS method is vastly superior to much more cumbersome clinical techniques, such as immunocytochemistry[19].

Reversed phase liquid chromatography, common in pharmaceutical analysis, was chosen as the inlet system because of the variety of analytes which could be accommodated. LC is also better suited for a number of common sample preparation techniques, such as solid phase extraction. The particle beam interface provides a straightforward way of introducing the sample into a quadrupole mass spectrometer. The interface is a very simple device, essentially it is a mildly heated vacuum assisted desolvation chamber. LC eluent entering the desolvation canister is converted to an aerosol using helium. In the desolvation canister the eluent rapidly evaporates leaving the lower volatility sample as a particle dispersion in the helium and gas phase solvent. The sample particles and gas phase solvent form a jet as they are sprayed out of the other end of the desolvation canister into a partial vacuum. The rapidly expanding gas phase solvent is separated from the sample particles using two skimmer cones. The process results in the sample particles entering the ion source where they are vaporized and ionized. This can result in the production of classical EI spectra which can be matched against entries in a spectral library to aid in sample identification. The system can also be operated in the CI mode. Using a combination EI/CI source the analyst can switch from one ionization technique to the other in a relatively short time. Chromatographic parameters are also retained. This might not be the case if a different "soft" ionization technique, such as FAB or electrospray, were employed.

MATERIALS AND METHODS

The equipment included a TRIO 2000 single quadrupole mass spectrometer equipped with a combination EI/CI source (VG Biotech Division of Fisons Instruments, Altrincham UK). The liquid chromatograph was a Model 1050 pump, autosampler and variable wavelength detector (Hewlett-Packard, Palo Alto CA USA). MS control and spectral processing was via MassLynx™ release 1.5 (VG Biotech). MassLynx™ functions in a Windows™ 3.1 environment (Microsoft Corporation, Redmond WA USA) on a 486/66 MHz PC. Separations were performed using a 2.0 x 300 mm μBondapak C_{18} column (Waters Division of Millipore, Milford MA USA). B&J Brand™ high purity acetonitrile was purchase from Burdick and Jackson (Baxter Healthcare Corporation, Muskegon MI USA). Distilled deionized water

was provided by a NANOpure II water purification system (Barnstead Thermolyne, Dubuque IA USA). Research purity methane was used as the reagent gas for chemical ionization (Matheson, Bridgeport NJ USA). Samples of aprobarbital, butabarbital, and butethal were purchase from Sigma Chemical Co. (St. Louis MO USA). All other barbiturates were provided by Ganes Chemicals (Pennsville NJ USA, see Figure 1 for barbiturate structures).

The mobile phase used was 70/30 water/acetonitrile adjusted to pH 3.0 using formic acid. The mobile phase was filtered and degassed by sonication under vacuum. Samples of barbiturates were prepared at approximately 0.1 mg/ml in mobile phase. UV detection (220 nm) was performed in series with the mass spectrometer. Analyses were conducted at ambient temperature using a 5 µl injection volume. Flow rate was 270 µl/min.

For EI+ spectra, the EI source was tuned using a sample of butalbital (although any of the barbiturates will suffice). Comparison of the "tuned" spectra to an entry in the NIST library was performed to determine accuracy. For CI+ spectra, appropriate reagent gas pressure was determined by monitoring the m/z 19 and m/z 29 peaks. When a 1:1 ratio between the peaks was attained, further tuning of the source was performed using butabital and monitoring for the presence of the molecular ion at M+H. Tuning was always performed using the LC as the inlet system, and the mobile phase as the sample eluent.

RESULTS AND DISCUSSION

Figure 2 is a list of the EI+/CI+ spectra of the barbiturates. Positive ion MS was selected because of its compatibility with the acidic mobile phases commonly encountered in reversed phase HPLC. The CI+ spectra (right column) show the clear base peak generated by the molecular ion adduct M+H. Barbiturates are generally distinguished by the identities of the R-groups at the #5 carbon. Determining the molecular weight of an unknown disubstituted barbituric acid is critical in establishing identity.

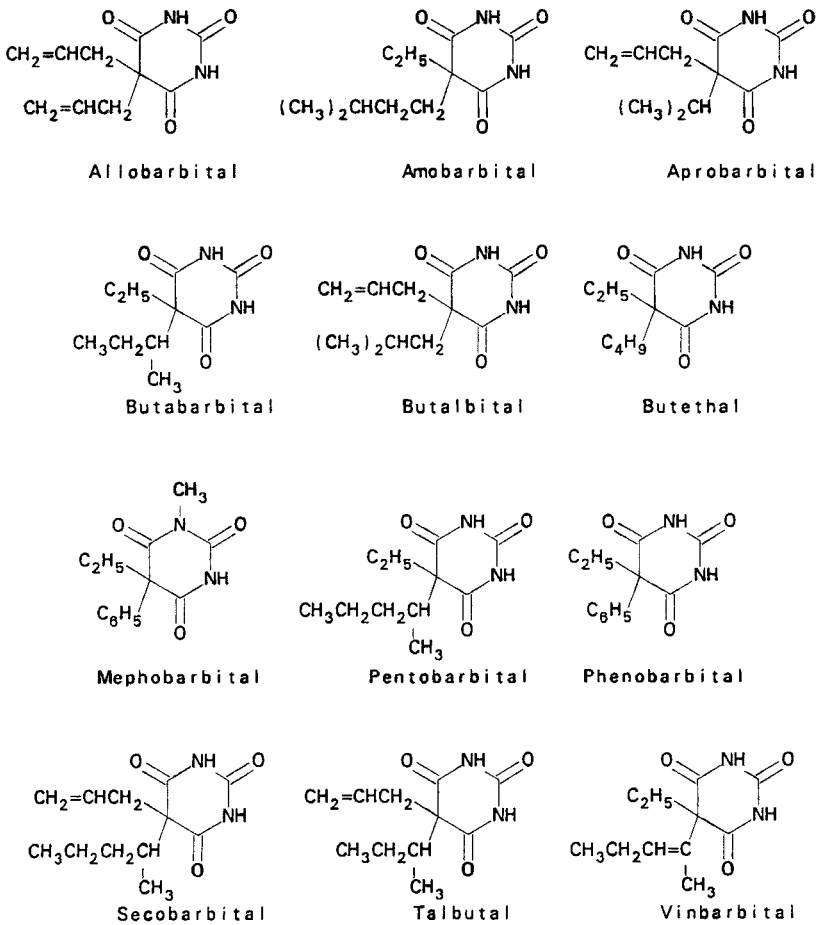


FIGURE 1
Barbiturate Structures

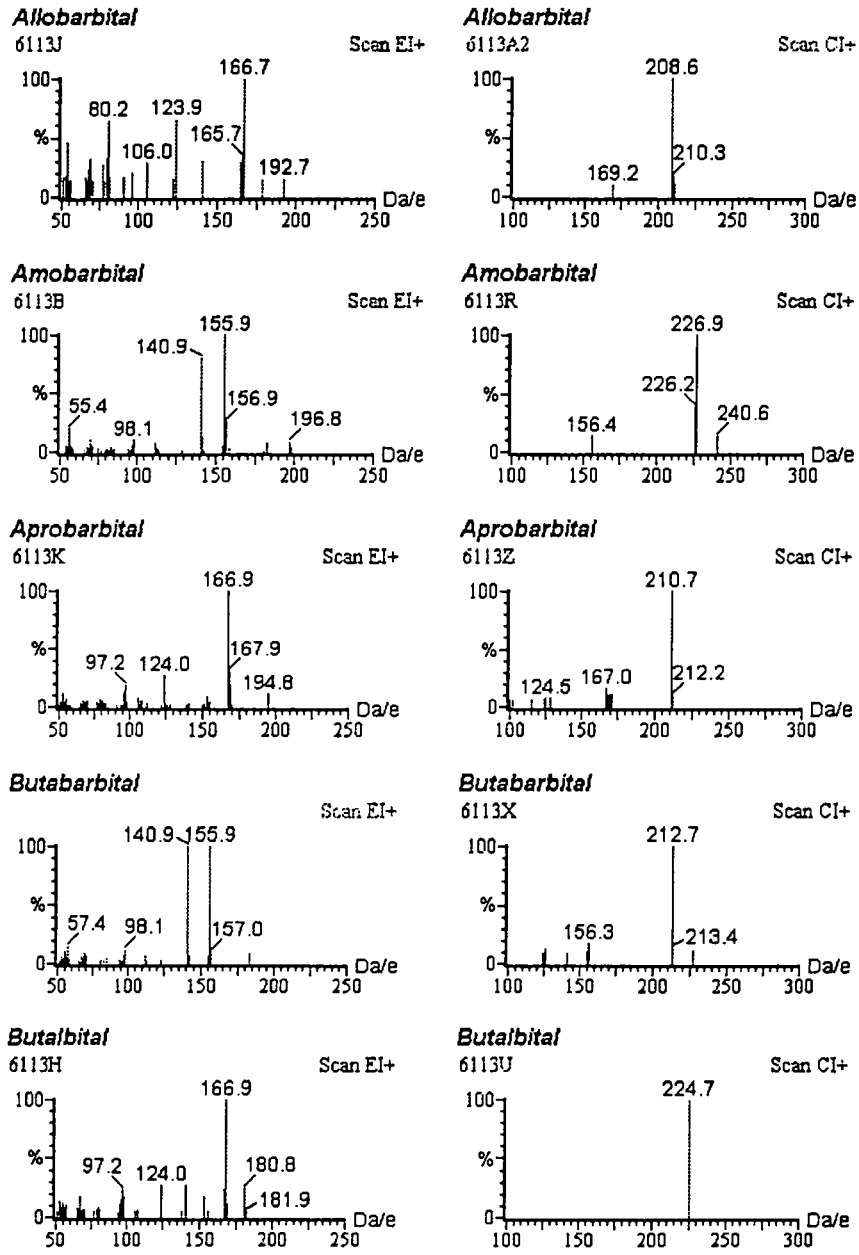
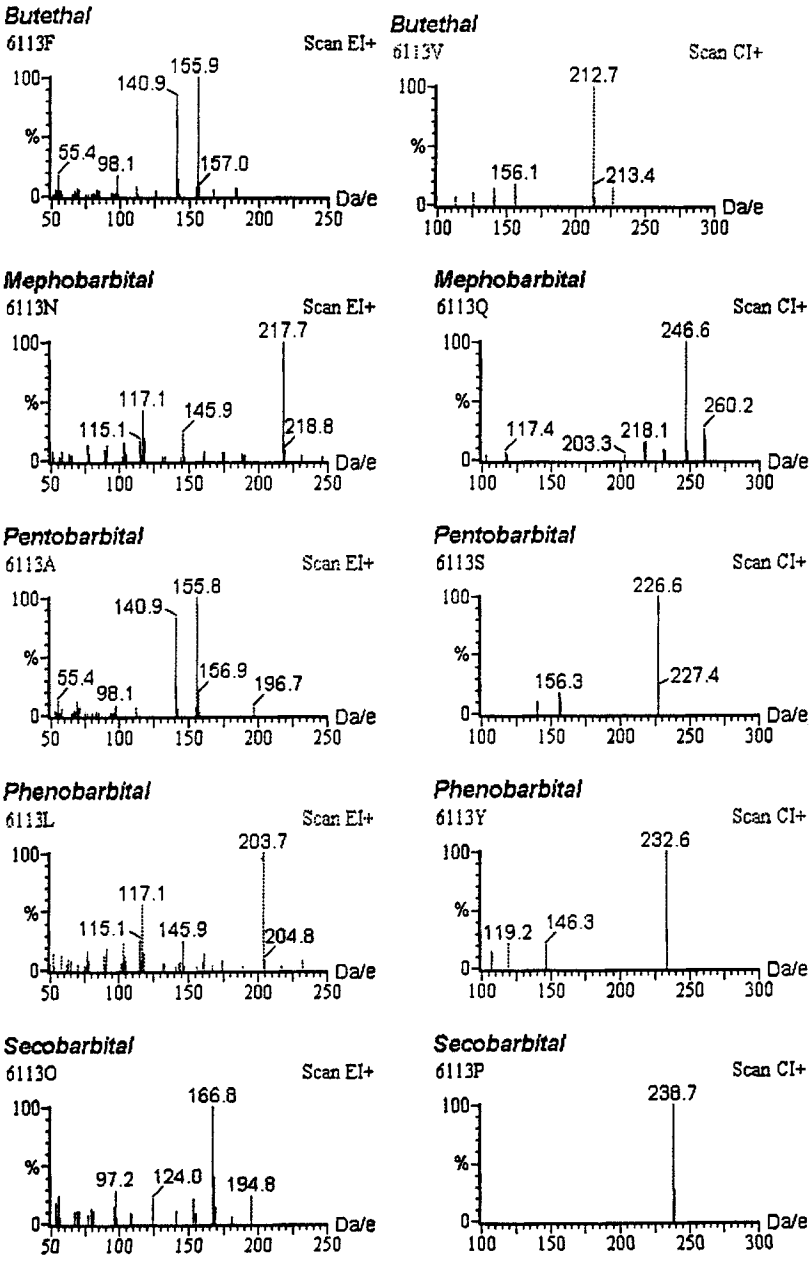


FIGURE 2

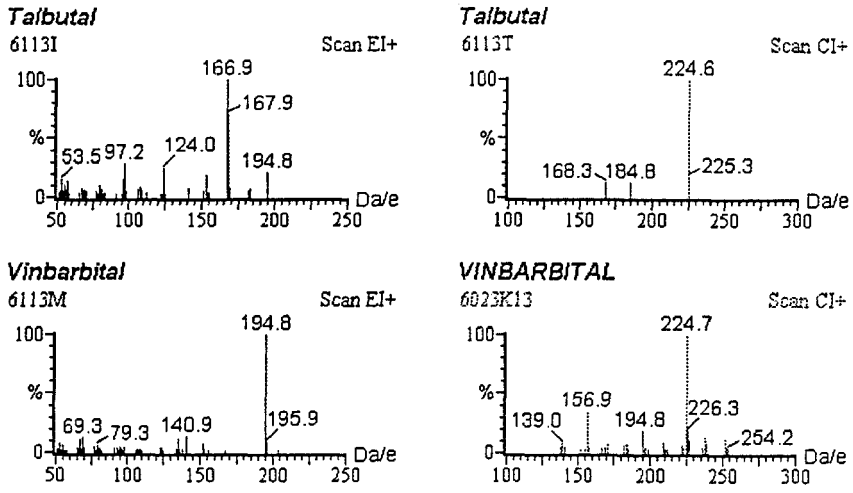
Barbiturate EI/CI Mass Spectra

FIGURE 2 (cont'd)

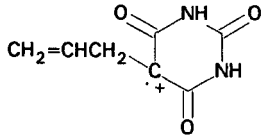


(cont'd)

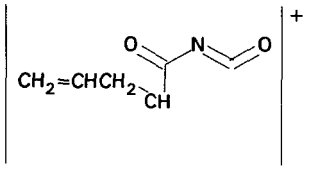
FIGURE 2 (cont'd)



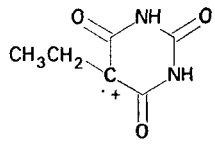
The highly similar EI+ spectra of allobarbital, aprobarbital, butalbital, secobarbital, and talbutal are due to the preferential loss of the alkyl R-groups while the ally R-group (CH_2CHCH_2-, present in all five) is retained along with the intact pyrimidinetrione ring. This generates the base peak at m/z 167, representing the following structure:



Another consistent ion present for these compounds is at m/z 124, caused by ring cleavage and subsequent rearrangement yielding:



Another common base peak observed in the EI+ spectra is at m/z 156, found in amobarbital, butabarbital, butethal, and pentobarbital. In this case the fragment ion is generated due to the preferential loss of the larger alkyl R-group, and the retention of C_2H_5- yielding the following structure:



Along with m/z 156, an equally strong ion is observed at m/z 141, and would be consistent with the loss of CH_3 from the remaining R-group. Other base peaks observed in the EI+ spectra of the remaining barbiturates conform to the same pattern of R-group elimination. In vinbarbital the base peak is at m/z 195 generated by the loss of the alkyl R-group C_2H_5-, again illustrating the preferential loss of an alkyl R-group over an allyl R-group. Phenobarbital exhibits a base peak at m/z 204, consistent with the loss of the alkyl R-group C_2H_5- while the phenyl ring is retained. The same is true for mephobarbital (the higher mass is due to the methylated nitrogen at position #1).

From Table 1, 3 pairs of barbiturates are observed to possess identical base peaks in both the EI+ and CI+ spectra. When a situation like this is encountered, careful evaluation of both spectral and retention information will provide the identity of the eluting compounds. Using the combination of the techniques, the analyst can accurately identify co-eluting multicomponent mixtures as long as the spectra are distinct. This approach has previously been demonstrated using UV photodiode array[20]. Using mass spectroscopy the EI+ spectra is examined for specific mass peaks, i.e., mass peaks that are present exclusively in spectra of one compound, but absent in the spectra of a near or co-eluting compound. Accurate selection of such an identifier peak is in a specific mass spectra is critical. The peak is usually a minor peak (a major peak with an intensity approaching that of the base peak would by definition provide the analyte with a distinct spectra). Spectral processing will compensate for most background elements, but the analyst should observe if a specific low intensity peak is truly part of the spectra, or if it is

TABLE 1
BARBITURATE MASS SPECTRA BASE PEAK SUMMARY

Sample	Molecular Weight* (amu)	Base Peak CI+* (m/z)	Base Peak EI+* (m/z)
Allobarbital	208	209	167
Aprobarbital	226	227	156
Amobarbital	210	211	167
Butabarbital	212	213	156
Butalbital	224	225	167
Butethal	212	213	156
Mephobarbital	246	247	218
Pentobarbital	226	227	156
Phenobarbital	232	233	204
Secobarbital	238	239	167
Talbutal	224	225	167
Vinbarbital	224	225	195

*Rounded to nearest whole number.

noise. Typically this is determined by monitoring the presence of the peak throughout the chromatogram, and noting if the relative abundance jumps sharply when the peak elutes. Figure 3 is the analysis of a sample consisting of equal parts butabarbital and butethal. The UV analog signal shows baseline resolution of the compounds. The TIC shows the broader elution profile associated with peaks as they pass through the desolvation canister of the particle beam. Although not completely resolved, accurate spectra of both compounds can be acquired from this analysis. Also shown is the scan of m/z 167, a mass peak exclusive to butethal. If a less complete resolution had occurred, this would distinguish the identities. Figure 4 is an analysis of butalbital and talbutal. Again the UV analog shows close to baseline resolution of the compounds, but the TIC shows a profile that is poorer than the previous example. In this case, the presence of a mass peak at m/z 195 in the talbutal spectra provides the means of identification. Figure 5 is an example of a true worst case scenario. Amobarbital and pentobarbital are incompletely resolved chromatographically. UV-photodiode array has proven to be extremely accurate at resolving the non-specific spectra of barbiturates[1,2], but this discriminating capability does require that the peaks be chromatographically resolved. In a situation like this UV-photodiode array would be useful in indicating

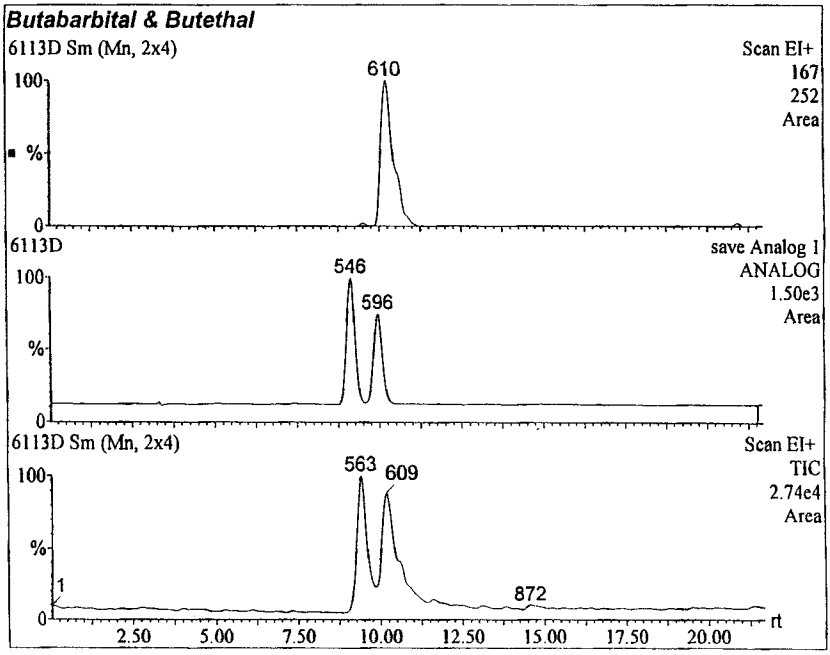


FIGURE 3

Detection of Butabarbital and Butethal Using EI+ Mass Spectroscopy (Specific detection of butethal at m/z 167)

the presence of a co-eluting compound via peak purity determination. Likewise, UV-photodiode array with multicomponent analysis would require a sharper spectral distinction[20] for accurate identification, although it would also indicate problems with eluting peak purity. The TIC shows the peaks merged into one another, the only way to discriminate the two under these circumstances is to monitor for the presence of the fragment ion at m/z 183, found exclusively in amobarbital.

CONCLUSION

The use of HPLC-particle beam EI/CI mass spectroscopy as a qualitative identification technique for barbiturates was successful. For the most part,

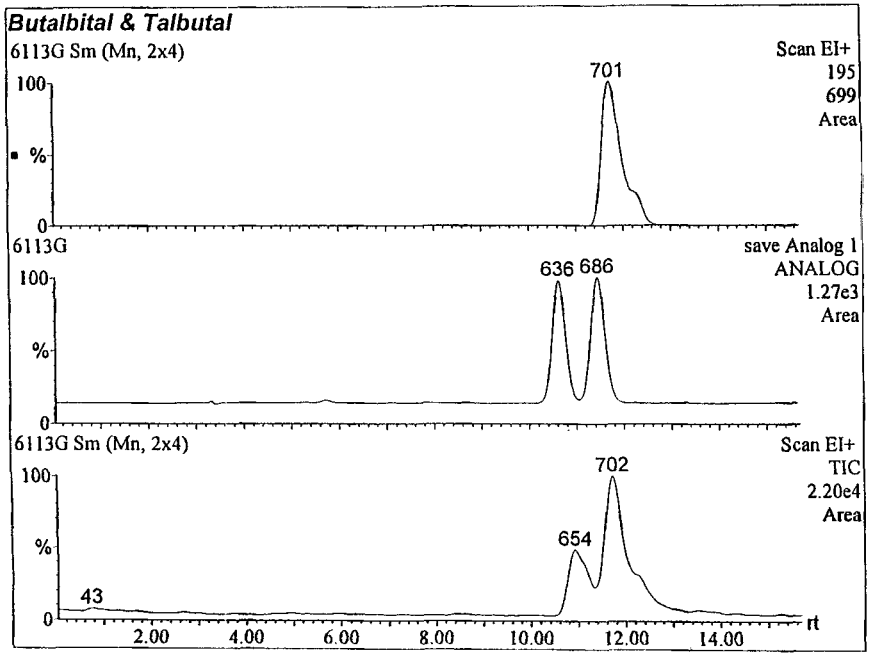


FIGURE 4
Detection of Butalbital and Talbutal Using EI+ Mass Spectroscopy (Specific detection of talbutal at m/z 195)

barbiturates were accurately identified by comparison of the EI and CI spectra. In those cases where spectra are very similar additional characterization via retention differences can be incorporated to enhance peak identification. The use of "identifier" mass peaks in similar spectra proved an accurate tool when chromatographic resolution was incomplete. The use of the particle beam interface should make the technique applicable not only in the pharmaceutical manufacturing or in related clinical assays, but in any industry where reversed phase HPLC is common. Two deficiencies in the technique were noted during the course of experimentation. The first was the sample band broadening associated with the particle beam interface. While representative spectra could be acquired from incompletely resolved peaks (such as Figure 3 and 4), this would

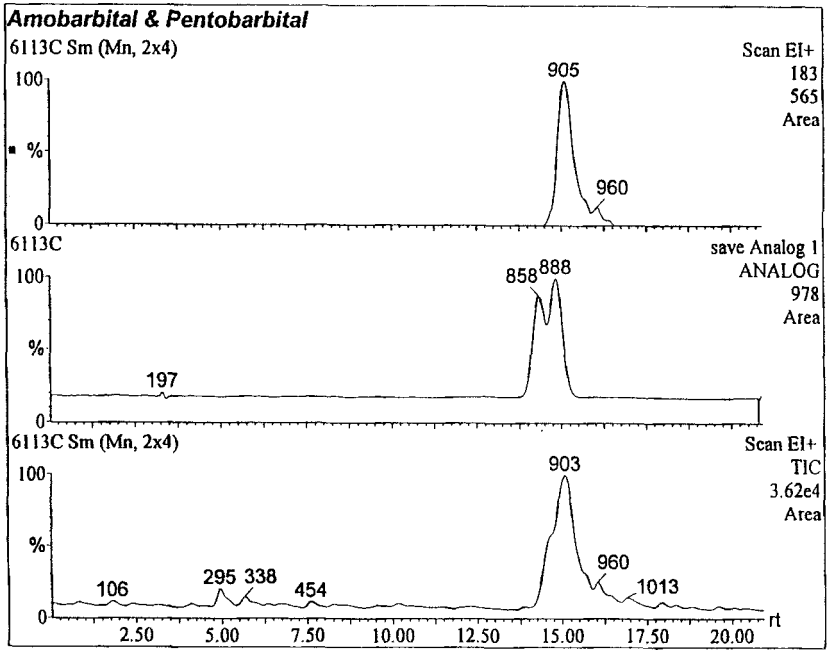


FIGURE 5

Detection of Amobarbital and Pentobarbital Using EI+ Mass Spectroscopy (Specific detection of amobarbital at m/z 183)

limit the technique if applied in a quantitative aspect. Of course, sample resolution could be enhanced by the use of a weaker mobile phase. However, this would extend analysis time and degrade peak shape which would cause a loss in sensitivity and potentially the generation of a non-representative spectra. Also, while not an analytical deficiency, LC-MS tends to increase routine instrument maintenance. LC-MS, particularly LC-CI, is an extremely aggressive technique. Cleaning the EI/CI source was typically carried out on a weekly basis. This in turn increases the wear on the source, and the likelihood that the source might be damaged. While random accidents cannot be avoided, impact on productivity can be limited by appropriate maintenance scheduling.

Further investigations into the technique will focus on quantitative as well as qualitative analyses, and also in establishing minimum concentrations at which the barbiturates can be accurately identified.

REFERENCES

1. Ryan, T.W., J. Liq. Chromatogr., 16 (1) 1993.

2. Ryan, T.W., J. Liq. Chromatogr., 16 (2) 1993.

3. Thorman, W., Merser, P., Marcolli, C., Binder, F., J. Chromatogr., 545 (2) 1991.

4. Ryan, T.W., "Determination of Mono or Di-Substitution in Barbituric Acids by UV Photodiode Array", Ganes Chemicals internal report, 3/6/92.

5. White, P.C., J. Chromatogr., 200, 1980, pp. 271-276.

6. Van Langenhouse, A., Biller, J.E., Biemann, K., Brown, T.R., Biomed Mass Spectrom., 9 (5) 1992.

7. Benchekroun, Y., Ribon, B., Desage, M., Brazier, J.L., J. Chromatogr., 31 (2) 1986.

8. Barbour, A.D., J. Anal. Toxicol., 15 (4) 1991.

9. Prakash, C., Adedoyin, A., Wilkinson, G.R., Blair, I.A., Biol. Mass Spectrom., 20 (9) 1991.

10. Kudo, K., Nagata, T., Kimura, K., Vehori, R., Noda, M., Forensic Sci. Int., 37 (3) 1988.

11. Villen, T., Petters, I., J. Chromatogr., 258, 1993, pp 267-270.

12. Zukowski, J., Sybilska, D., Bojarski, J., Szejtli, J., J. Chromatogr., 436 (3) 1988.

13. Reif, V.D., Kaufmann, K.L., DeAngelis, N.J., Frankhouser, M.C., J. Pharm Sci., 75 (7) 1986.

14. Rop, P.P., Spinazzola, J., Zahra, A., Bresson, M., Quicke, J., Viala, A., J. Chromatogr., 427 (1) 1988.

15. Haganaka, J., Wakai, J., J. Chromatogr., 390 (2) 1987.

16. Mangin, P., Lugnier, A.A., Chaumont, A.J., J. Anal Toxicol., 11 (1) 1987.

17. Jones, L.V., Whitehouse, M.J., Biomed Mass Spectrom., 8 (5) 1981.

18. Gill, R., Moffat, A.C., Smith, R.M., Hurdley, T.G., J. Chromatogr. Sci., 24 (4) 1986.

19. Ishiyama. I., Mukaida, M., Tanabe, R., Kaiho, M., Ueyama, M., J. Forensic Sci., 32 (5) 1987.

20. Ryan, T.W., J. Liq. Chromatogr., 16 (7) 1993.

Received: July 30, 1993
Accepted: August 25, 1993