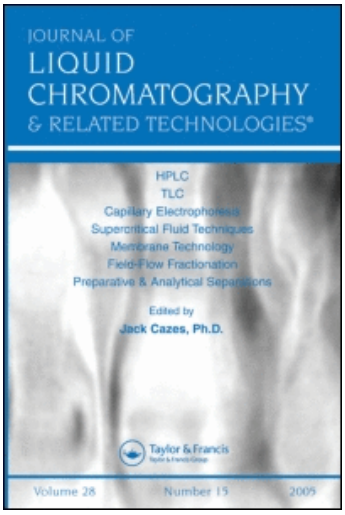

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Simultaneous Determination and Separation of Several Barbiturates and Analgesic Products by Ion-Pair High-Performance Liquid Chromatography

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SIMULTANEOUS DETERMINATION AND SEPARATION OF SEVERAL BARBITURATES AND ANALGESIC PRODUCTS BY ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A sensitive, rapid, and accurate high-performance liquid chromatographic method for the simultaneous determination of several barbiturates and analgesic drugs is described. Reversed-phase ion-pair chromatography, using 1-decanesulfonic acid sodium salt as the counterion, was used. The method is rapid because no sample extraction is involved. Internal standards were used for the quantification. A μ Bondapak C_{18} column with a mobile phase of 0.01M KH_2PO_4 and acetonitrile (7:3) pH 3.2 for barbiturates and (1:1) pH 7.9 for propoxyphene HCl or napsylate was used at a flow rate of 1.0 mL/min. The barbiturates and propoxyphene compounds were monitored at 214 nm and 220 nm respectively. Precision and ruggedness (relative standard deviation) were below 1.0%. The detection limit ranged from 0.05 μg/mL to 0.1 ng/mL, depending on the compound. The method is stability indicating and can be applied to bulk pharmaceutical chemicals.

INTRODUCTION

Barbiturates are organic compounds generally used as sedative, hypnotic, and anticonvulsant drugs, or as adjuncts in anesthesia.

Methods for analyzing barbiturates are numerous. They have been determined colorimetrically (1), by polarography (2,3), by potentiometric titration (4-10), by UV spectrophotometry (11-16), identified by thin-layer chromatography (TLC) (17-19) and quantified by densitometry (20-22).

The separation and quantitative determination of certain barbiturates by gas-liquid chromatography (GLC) using different detectors (23-30), and their simultaneous determination by gas chromatography/mass spectrometry (GC/MS), have been described (31-33).

Although many of these GLC methods are sensitive and accurate they require lengthy derivatization procedures. Problems due to breakdown of these barbiturates at higher temperatures may also occur.

Several high-performance liquid chromatographic (HPLC) methods have been described for determining barbiturates and analgesic drugs (34-42).

Many of the published methods address the detection of drugs in biological fluids, and are not particularly suited to the problems (such as detection of by-products, unreacted starting materials, and kinetic studies) encountered in bulk pharmaceutical production.

Potentiometric and gravimetric methods described in the USP-NF and BP compendia for determining pentobarbital and secobarbital and their corresponding sodium salts are tedious, time-consuming, and not sensitive.

The HPLC method described in this paper permits the separation and quantification of several barbiturates and analgesic drugs in pharmaceutical production, including wet cake, mother liquor and reaction completion samples, without prior purification or extraction.

EXPERIMENTAL

Apparatus

The system was a Varian 5000 liquid chromatograph with an 8055 autosampler (both by Varian Instrument Division, Walnut Creek, CA) and a 783A programmable absorbance detector by Applied Biosystems, Foster City, CA. The diode array instrument was a Model 9010 pump, a Model 9095 autosampler and a Model 9065 photodiode array detector (all by Varian Instrument Division). System control was via the Series 9020 LC Star Workstation, Revision C (Varian Instrument Division). Spectral manipulation and comparison were performed using Polyview™ Revision D (Varian Instrument Division). The LC control and spectral processing software functions in a Windows™ environment (Windows Verson 3.0, Microsoft Corporation, Redmond, WA) on a 386 PC equipped with a math coprocessor. A 300 x 3.9 mm μ Bondapak C_{18} column (Waters Division of Millipore, Milford, MA) was used.

Reagents

High purity acetonitrile was purchased from Burdick and Jackson (Baxter Healthcare Corporation, Muskegen MI). Deionized water was *from a*

NANOpure II (Barnstead Thermolyne, Dubuque IA) water purification system. Acetoxy (α-*d*-2-acetoxy-4-dimethylamino-1,2-diphenyl-3-methylbutane), carbinol hydrochloride (α-*d*-4-dimethylamino-1,2-diphenyl-3-methyl-2-butanol hydrochloride), propoxyphene HCl, propoxyphene napsylate and all the barbiturates were purchased from US Pharmacopeial Convention Inc., Rockville, MD. Methylparaben (methyl 4-hydroxybenzoate, 99%), ethylparaben (ethyl 4-hydroxybenzoate, 99%), DSA (1-decanesulfonic acid sodium salt, 99%) and papaverine HCl, 99%, were purchased from Aldrich Chemical Company, Milwaukee, WI. Pentobarbital analog [5-(1,3-dimethylbutyl)-5-ethylbarbituric acid] and secobarbital analog [5-(1,3-dimethylbutyl)-5-(2-propenyl)barbituric acid] were provided by Ganes Chemicals, Inc., Pennsville NJ.

Determination of Barbiturates

The mobile phase consisted of aqueous solution (0.01M KH_2PO_4 - 0.005M DSA) and acetonitrile (7:3). The pH of the mobile phase was adjusted to 3.2 with phosphoric acid (85%). After mixing and pH adjustment the mobile phase was filtered through a 0.22-μm Millipore filter and degassed by sonication under vacuum. Methylparaben and ethylparaben were used as internal standards, and were prepared in the mobile phase (0.2 mg/mL). Barbiturate standards or samples were prepared to contain 0.5 mg/mL in the internal standard solution. Ethylparaben was used as an alternate internal standard if methylparaben had a retention time close to that of a barbiturate.

TABLE 1

Precision and Ruggedness Analyses

	Precision, RSD (%) n=5		Ruggedness, RSD (%) n=5	
	Day 1	Day 2	Day 1	Day 2
Pentobarbital	0.07	0.06	0.09	0.14
Secobarbital	0.09	0.16	0.75	0.33
Phenobarbital	0.45	0.92	0.66	0.83
Propoxyphene base	0.12	0.96	0.38	0.60
Acetoxy	0.26	0.78	0.53	0.64
Carbinol HCl	0.04	0.10	0.74	0.44

The liquid chromatograph was equipped with a 214 nm detector and the flow rate was about 1 mL/min. As an example, the retention times for methylparaben, pentobarbital, and secobarbital were about 7.2, 10.7, and 13.7 minutes, respectively. The standard preparation was injected several times (10 μL) and the response for the barbiturate and the internal standard was measured. The relative standard deviation for five replicate injections of the standard preparation in the ratios of the peak area of the barbiturate to that of the internal standard is shown in Table 1. The sample preparation was injected and the concentration of the barbiturate in the mixture was determined.

Determination of Propoxyphene HCl or Napsylate

The mobile phase was prepared by mixing 500 mL of an aqueous solution (0.01M KH_2PO_4-0.005M DSA) with 500 mL acetonitrile, 1 mL of heptylamine was added and the pH of the solution was adjusted to 7.9 with phosphoric acid

(85%). After mixing and pH adjustment the mobile phase was filtered through a 0.22-μm Millipore filter and degassed by sonication under vacuum. Papaverine HCl was used as internal standard and was prepared in the mobile phase (0.2 mg/mL). Propoxyphene HCl (or napsylate) standards or samples were prepared to contain 1 mg/mL in the internal standard solution. The liquid chromatograph was equipped with a 220-nm detector and a 300 x 3.9 mm μ Bondapak C_{18} column. The flow rate was about 2 mL/min and the injection volume was about 10 μL. As an example the retention times for papaverine HCl, acetoxy, carbinol HCl, and propoxyphene base were about 2.7, 10.1, 11.9, and 13.6 minutes, respectively. Using 1.0 mL/min, the retention times for papaverine HCl, acetoxy, carbinol HCl, and propoxyphene base were about 5.5, 11.7, 13.2, and 17.4 minutes, respectively. The relative standard deviation for five replicate injections of the standard preparation in the ratios of the peak area of each of these compounds to that of the internal standard is shown in Table 1. The sample preparation was injected into the chromatograph and the concentrations of each of these compounds in the mixture was determined.

Results and Discussion

Several barbiturates were completely separated using the above mobile phase and a μ Bondapak C_{18} column as shown in Fig 1. Because of its high volatility, low viscosity, and UV transparency at short wavelength, acetonitrile was chosen over methanol in preparing the mobile phase. Silica stationary

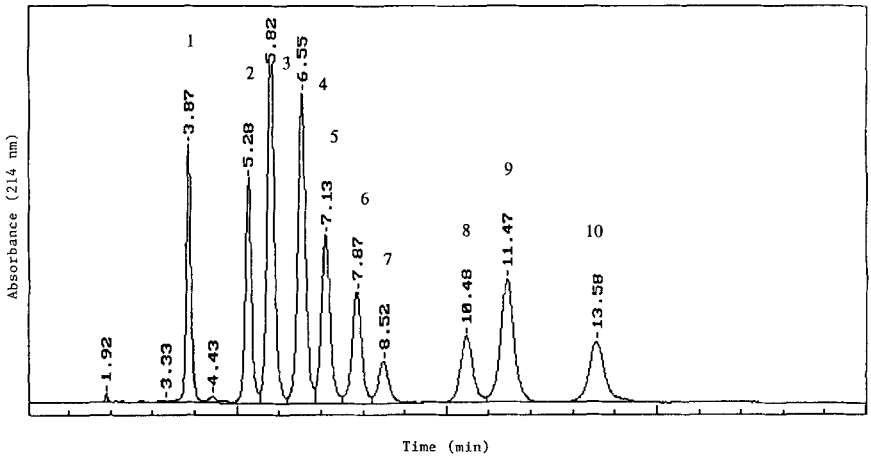

Fig. 1. Separation of barbital (1), allobarbital (2),aprobarbital (3), phenobarbital (4), vinbarbital (5), butalbital (6), talbutal (7), pentobarbital (8), mephobarbital (9), and secobarbital (10). Column, 300 x 3.9 mm μ Bondapak C_{18}, flow rate 1.0 mL/min, detection at 214 nm.

phase supports with C_{18} bonded phases provided good efficiency and offered satisfactory selectivity for a wide range of these compounds at lower pH. However, phenyl and CN stationary phases gave poor stability and short column lifetimes at low pH.

Specificity or selectivity of this method was tested to show that it was capable of measuring accurately and without bias the analyte of interest in the presence of other likely components. Complete resolution of pentobarbital and secobarbital from their potential homologs, with good peak symmetry and no apparent shoulders, was demonstrated as shown in Fig 2. Chromatographic purity at peak heart was also determined using the diode array instrument over 205nm-254nm. This technique is applied to resolve coeluting peaks from the

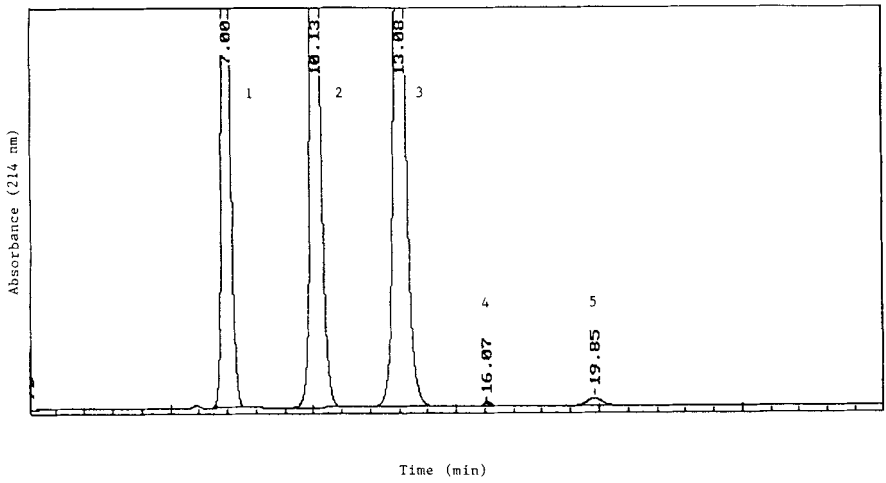

Fig 2. Chromatogram of methylparaben (1), pentobarbital (2), secobarbital (3), pentobarbital analog (4), secobarbital analog (5). Column, 300 x 3.9 mm μ Bondapak C_{18}, flow rate 1 mL/min, detection at 214 nm.

products. The plot reports (Fig 3) showed that pentobarbital, secobarbital, and propoxyphene base each exhibited a single peak with no other impurity peaks embedded in them. Barbital, phenobarbital, butalbital, and secobarbital were simultaneously separated and quantitatively determined in one blend using ethylparaben as internal standard as shown in Fig 4. Also a blend of pentobarbital and secobarbital was analyzed quantitatively using methylparaben as internal standard as shown in Fig 2.

The GC method for determining the two related compounds (acetoxy and carbinol HCl) in propoxyphene HCl or napsylate described in the USP gave poor resolution and high RSD values. Following the above HPLC procedure these analogs were completely resolved and determined quantitatively using papaverine HCl as internal standard, as shown in Fig 5.

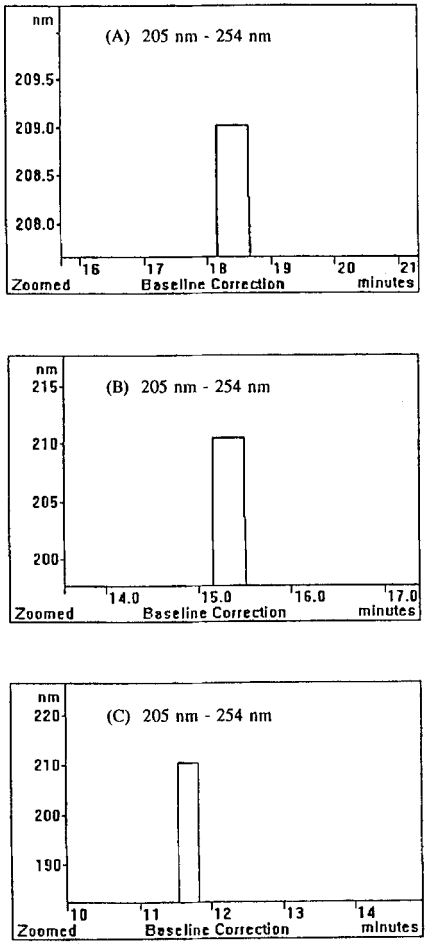

Fig 3. Peak purity at peak heart for propoxyphene base (A), secobarbital (B), and pentobarbital (C), performed over 205 nm - 254 nm using diode array spectrophotometry.

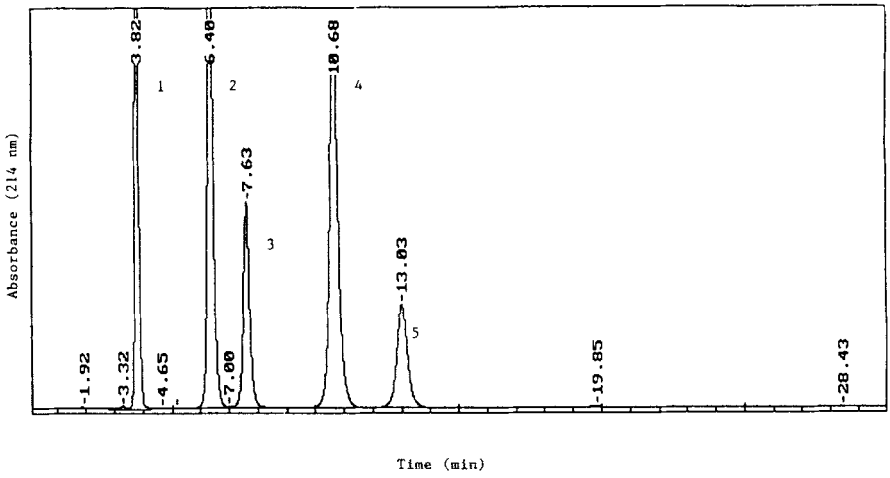

Fig 4. Chromatogram of barbital (1), phenobarbital (2), ethylparaben (3), butalbital (4), and secobarbital (5). Column, 300 x 3.9 mm μ Bondapak C_{18}, flow rate 1 mL/min, detection at 214 nm.

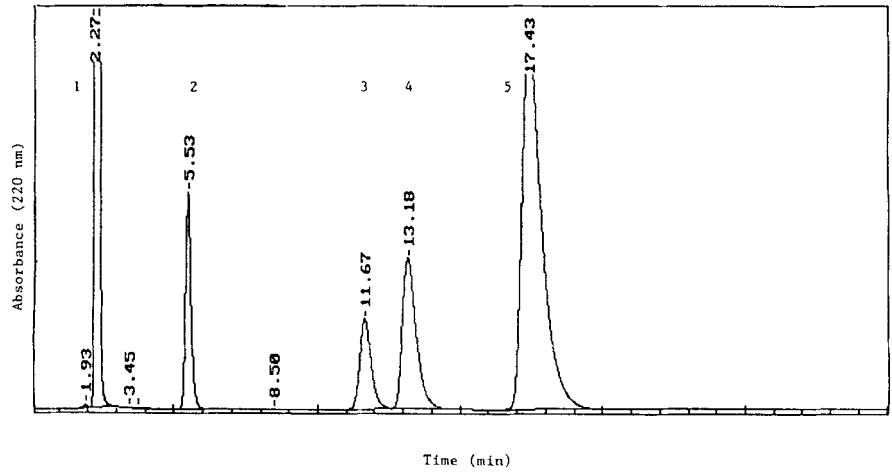

Fig 5. Chromatogram of napsylate (1), papaverine HCl (2), acetoxy (3), carbinol HCl (4), and propoxyphene base (5). Column, 300 x 3.9 mm μ Bondapak C_{18}, flow rate 1 mL/min, detection at 220 nm.

Precision and ruggedness studies were performed on several of these compounds by two different chemists on different days using different columns; the RSD's from each chemist (n=5) are listed in Table 1.

Linearity of response was measured for pentobarbital, secobarbital and propoxyphene napsylate at 6 levels. The area count of each compound plotted against its consecutive concentration produced a straight line through the origin. Regression analysis yielded a correlation coefficient (RS) of 0.9992 for pentobarbital, 0.9993 for secobarbital, and 0.9998 for propoxyphene base. These results showed a high degree of linearity over a range of up to 110%.

Recovery study was performed by dissolving known amounts of several drugs at various levels, and determining the concentration of each drug using the recommended procedure. Results are summarized in Table 2.

The lower limit of detectability for pentobarbital, secobarbital and propoxyphene base was 0.05 μg/mL. For acetoxy and carbinol HCl the limit was 0.2 μg/mL, and for phenobarbital and mephobarbital it was 0.1 ng/mL.

To explore the utility of this method two experiments were performed: 1) A solution of each drug (0.5 mg/mL in its mobile phase) was exposed to ultraviolet (254 nm and 366 nm) light for 30 hours. 2) Solutions as above were kept at 50°C for 2 days. The degraded samples were then analyzed using the above HPLC method. The two experiments gave very similar results for each compound, so only one chromatogram from each set is shown. Several decomposition products were completely resolved from their parent compounds, as can be seen for pentobarbital and its sodium salt (Fig 6),

TABLE 2

Recovery Study

Compound	μg Added	μg Found	% RSD	% Recovered
Pentobarbital	3.05	3.10	0.06	101.6
	5.15	5.05	0.14	98.0
	8.38	8.60	0.27	102.6
	9.22	9.40	0.21	102.0
	10.06	10.06	0.05	100.0
	11.12	11.08	0.10	99.6
Secobarbital	3.21	3.20	0.89	99.7
	5.19	5.20	0.02	100.2
	8.25	8.35	0.37	101.2
	9.08	8.95	0.10	98.6
	10.03	9.90	0.42	98.7
	11.21	11.21	0.45	100.0
Propoxyphene Base	3.00	3.05	0.10	101.7
	5.13	5.15	0.55	100.4
	8.11	8.11	0.29	100.0
	9.06	9.10	0.48	100.4
	10.01	10.04	0.59	100.3
	11.28	11.20	0.05	99.3

n=2 for each analysis

secobarbital and its sodium salt (Fig 7), and propoxyphene HCl and propoxyphene napsylate (Fig 8). The degraded samples were also subjected to diode array analysis, and the plot reports showed that the pentobarbital, secobarbital, and propoxyphene base peaks had no other impurity peaks embedded in them (Fig 3). This illustrates that the method may be used for determining the stability of propoxyphene HCl, propoxyphene napsylate, pentobarbital and secobarbital (and their sodium salts) in bulk pharmaceuticals.

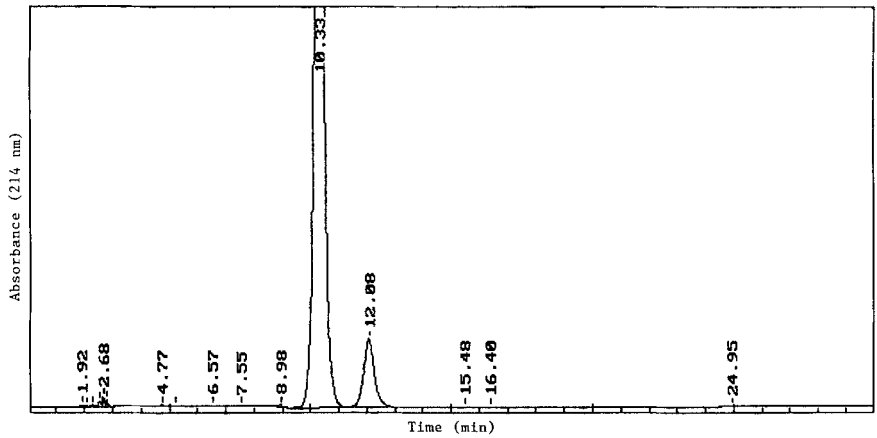

Fig 6. Chromatogram of a solution of pentobarbital exposed to ultraviolet light and heat. Column, 300 x 3.9 mm μ Bondapak C_{18}, flow 1.0 mL/min, detection at 214 nm.

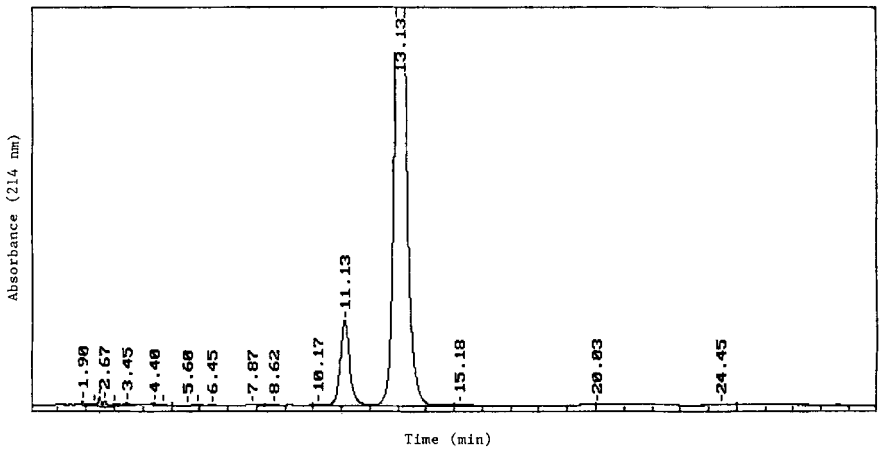

Fig 7. Chromatogram of a solution of secobarbital exposed to ultraviolet light and heat. Column, 300 x 3.9 mm μ Bondapak C_{18}, flow 1.0 mL/min, detection at 214 nm.

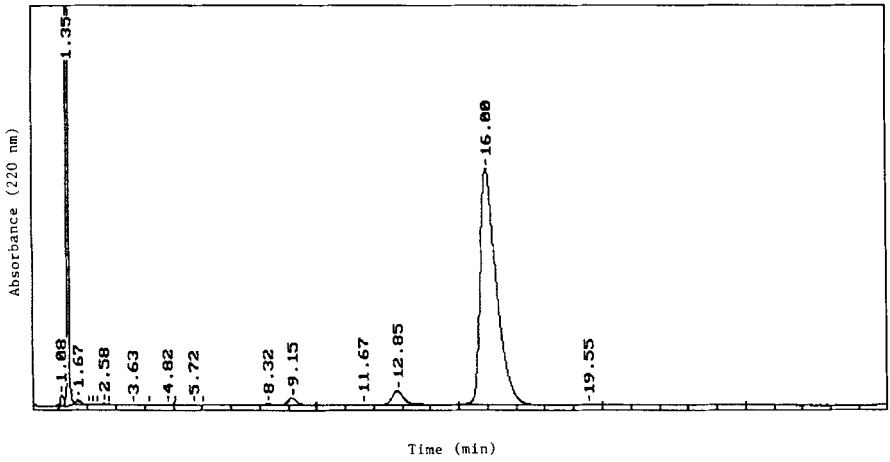

Fig 8. Chromatogram of a solution of propoxyphene napsylate exposed to ultraviolet light and heat. Column, 300 x 3.9 mm μ Bondapak C_{18}, flow 1.0 mL/min, detection at 220 nm.

The method is capable of detecting intermediates which might be found at trace levels in finished products. It can also be used for kinetic measurements to determine completion times for synthetic processes.

CONCLUSION

The method described provided sufficient sensitivity for detecting and quantitatively determining several barbiturates and propoxyphene compounds and their corresponding related compounds or analogs in bulk pharmaceuticals. The method was rapid because no extraction was involved, and precision was greatly improved as compared to other methods. It can be applied for the determination of propoxyphene HCl, propoxyphene napsylate, pentobarbital, secobarbital and their sodium salts in their bulk pharmaceutical productions.

The method may also be submitted to replace the potentiometric, gravimetric and GC methods for the determination of these drugs as described in the revised USP-NF.

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REFERENCES

1. H.M. Stevens, J. Forensic Sci. Soc., **19**: 199-201 (1979).
2. A. Temizer, Arch. Pharm. (Weinheim), **319**: 149-154 (1986).
3. W.F. Smyth, G. Svehla, P. Zuman, Anal. Chim. Acta, **52**: 129-138 (1970).
4. P.M. Coenegracht, H.J. Metting, D.A. Doornbos, Pharm. Weekbl. (Sci), **5**: 243-247 (1983).
5. G.D. Carmack, H. Freiser, Anal. Chem., **49**: 1577-1579 (1977).
6. G.M. Davis, J.E. Taphorn 3d, J.A. Caruso, J. Pharm. Sci., **63**: 1136-1138 (1974).
7. M.I. Blake, J. Hunt, H.J. Rhodes, J. Pharm. Sci., **63**: 89-91 (1974).
8. S.A. Soliman, Y.A. Beltagy, I.M. Roushdi, J. Pharm. Pharmocol., **21**: Suppl: 44S+ (1969).
9. M.S. Greenberg, B.J. Barker, J.A. Caruso, Anal. Chim. Acta, **54**: 159-161 (1971).
10. J.R. Monforte, W.C. Purdy, Anal. Chim. Acta, **52**: 25-33 (1970).
11. R. Guillen Sans, M. Guzman Chozas, Boll. Chim. Farm., **127**: 23-25 (1988).
12. R.G. Sans, M.G. Chozas, Arch. Pharm. (Weinheim), **321**: 65-68 (1988).

13. C.B. Walberg, Clin. Toxicol, **18**: 879-885 (1981).

14. A.M. Wahbi, M. Bararay, Analyst, **105**: 855-860 (1980).

15. M.B. Mrtek, J.E. Gearien, M.I. Blake, J. Pharm. Sci., **66**: 1019-1021 (1977).

16. F. DeFabrizio, J. Pharm. Sci., **66**: 811 (1977).

17. F. Fiorese, G. Vermuelen, C. Turcotte, Subst. Alcohol Actions Misuse, **3**: 61-76 (1982).

18. W. Bress, K. Ziminski, W. Long, T. Manning, L. Lukash, Clin. Toxicol., **16**: 219-221 (1980).

19. T.S. Vasundhara, D.B. Parihar, J. Chromatogr., **176**: 225-231 (1979).

20. S.S. Levin, M.F. Schwartz, D.Y. Cooper, J.C. Touchstone, J. Chromatogr., **154**: 349-352 (1978).

21. J.C. Touchstone, M.F. Schwartz, S.S. Levin, J. Chromatogr. Sci., **15**: 528-531 (1977).

22. Y. Garceau, Y. Philopoulos, J. Hasegawa, J. Pharm. Sci., **62**: 2032-2034 (1973).

23. W.M. Chow, B. Caddy, J. Chromatogr., **354**: 219-229 (1986).

24. T. Villen, I. Petters, J. Chromatogr., **258**: 267-270 (1983).

25. S. Dilli, A. Weekley, J. Chromatogr., **195**: 349-358 (1980).

26. H. Nau, D. Jesdinsky, W. Wittfoht, J. Chromatogr., **182**: 71-79 (1980).

27. D.B. Black, H. Kolasinski, E.G. Lovering, J.R. Watson, J. Assoc. Off. Anal. Chem., **65**: 1054-1058 (1982).

28. J.R. Sharman, J. Anal. Toxicol., **5**: 153-156 (1981).

29. C.E. Lau, M. Tang, J.L. Falk, Pharmacol. Biochem. Behav., **11**: 355-357 (1979).

30. S. Barkan, T.D. Doyle, J. Assoc. Off. Anal. Chem., **60**: 1022-1029 (1977).

31. K. Kuddo, T. Nagata, K. Kimura, R. Uehori, M. Noda, Forensic Sci. Int., **37**: 193-200 (1988).

32. Y. Benchekroun, B. Ribon, M. Desage, J.L. Brazier, J. Chromatogr. **420**: 287-296 (1987).

33. A. Van Langenhove, J.E. Biller, K. Biemann, T.R. Browne, Biomed. Mass Spectrom., **9**: 201-207 (1982).

34. W.F. Ebling, L. Mills-Williams, S.R. Harapat, D.R. Stauski, J. Chromatogr., **490**: 339-353 (1989).

35. G.K. Herkes, G.E. McKinnon, M.J. Eadie, J. Chromatogr., **496**: 147-154 (1989).

36. V. Shaukar, C. Damodaran, P.C. Sekharan, Forensic Sci. Int., **40**: 45-55 (1989).

37. V.D. Reif, K.L. Kaufmann, N.J. DeAngelis, M.C. Frankhouser, J. Pharm. Sci., **75**: 714-716 (1986).

38. P. Mangin, A.A. Lugnier, A.J. Chaumont, J. Anal. Toxicol., **11**: 27-30 (1987).

39. E. Sigel, F.A. Stephenson, C. Mamalaki, E.A. Barnard, J. Recept. Res., **4**: 175-188 (1984).

40. V. Das Gupta, J. Pharm. Sci., **73**: 1158-1160 (1984).

41. B. Levine, R. Blanke, J. Valentour, J. Anal. Toxicol., **7**: 207-208 (1983).

42. M.C. Bowman, L.G. Rushing, J. Chromatogr. Sci., **16**: 23-27 (1978).

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