Journal of Chromatography, 213 (1981) 337–339
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,963

Note

Fluorescence and UV detection of opiates separated by reversed-phase high-performance liquid chromatography

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(First received February 3rd, 1981; revised manuscript received May 4th, 1981)

Considering their importance in pharmacology, toxicology and neurobiology some of the physical properties of the classical (*i.e.*, non-peptide) opiates have lacked adequate investigation. In particular, their fluorescence properties, and the use of these for detection in liquid chromatography have been inadequately reported. On the other hand detection of some natural and synthetic opiates using either their intrinsic or derivatized fluorescence properties has been previously used in paper and thin-layer chromatography[1]. For liquid systems, however, the standard monograph on fluorescence work[2] implies that only reaction products of morphine have appreciable fluorescence. Handbooks have carried through the same information[3] which is repeated in a recent report where electrochemical detection of opiates is discussed[4]. Indeed, a computer search of the high performance liquid chromatographic literature shows that the sole mention of fluorescence detection of opiates was in a recent report where dextrorphan was detected[5]. In that report sensitivities were not mentioned.

Consequently, the purpose of this brief report is to examine some of the relevant properties of some of the more familiar opiate agonists and antagonists. We present a convenient separation and detection scheme and discuss the spectral and chromatographic parameters and give estimates of detection limits.

EXPERIMENTAL

High-performance liquid chromatography (HPLC) was performed on a Micromeritics Model 7000 B chromatograph which was equipped with a Model 785 variable-wavelength ultraviolet detector. The chromatograph was coupled to a Perkin-Elmer Model 204-S dual monochromator fluorescence detector operating in the ratio mode and using the Perkin-Elmer 20-μl detection cell. The source was a high-pressure xenon lamp. Initial fluorescence work on macroscopic samples was done with a Perkin-Elmer Model MPF-3 spectrofluorometer.

Chromatographic separations were worked out initially on a home packed 10-μm RP-18 column (25 cm × 4.6 mm) and the final separations reported here were done with a Supelco 15 cm × 4.6 mm LC-18 column.

All the opiate compounds were used in the form of their water soluble acid salts. Buffer and solvent compounds were of chromatographic grade filtered before

0021-9673/81/0000–0000/$02.50

use and water was from a Millipore "Milli-Q" purifier using house deionized water as its source.

RESULTS

Table I summarizes the UV absorbtion and fluorescence parameters of the opiates studied. We found that ionic strength was very important in developing good HPLC separations for these compounds. The best chromatographic separation parameters are given in Table II along with data on the effect of ionic strength on the separation.

TABLE I

UV AND FLUORESCENCE PROPERTIES OF OPIATES IN ACETONITRILE–PHOSPHATE BUFFER (pH 5.05) (50:50)

Fluorescence spectra taken at 10 μM concentrations.

Parameter	*Compound*			
	Morphine	*Etorphine*	*Naloxone*	*Levallorphan*
$\lambda_{max.}$ (nm)	285	289	280	280
$\varepsilon\lambda_{max.}$ (cm^2/mole)	1652	1807	1638	1062
ε_{255} (cm^2/mole)	14,000	20,400	10,140	3650
λ_{ex} (nm)	290	290	290	290
$\lambda_{ex}^{max.}$ (nm)	340	346	–	309
Rel. emission intensity	1	1.24	0.0	19.5

TABLE II

REVERSED-PHASE HPLC SEPARATION OF OPIATES ON SUPELCO LC-18 (15 cm × 4.6 mm) COLUMN

Flow-rate 2 ml/min. Eluent system: "strong": acetonitrile–phosphate buffer (pH 5.05) (65:35); "weak": acetonitrile–water (80:20).

	t_R *(min)*			
	Naloxone	*Morphine*	*Etorphine*	*Levallorphan*
Retention times, t_R, as a function of eluent composition:				
40% "strong"	1.9	2.3	2.7	7.4
55% "strong"	1.7	2.05	2.5	6.7
60% "strong"	1.7	1.9	2.45	6.5
Effect of ionic strength:				
Final concentration of buffer 3 m*M*		3.2	4.6	
Final concentration of buffer 4 m*M*		2.6	3.9	

DISCUSSION

Our major finding is that for optimal sensitivity detection monitoring of *both* UV absorption at 225 nm and fluorescence emission at 330 nm are desirable. The wavelength of 330 nm was chosen as the best compromise between the emission peaks of the three fluorescent opiates investigated. Studies on a single opiate may benefit from use of the maximum of the emission spectrum (Table I). For example, naloxone does not exhibit detectable fluorescence under our conditions but has a very high extinction coefficient at 225 nm. On the other hand, levallorphan fluoresces very strongly but has low extinction coefficients at 280 and 225 nm. The lowest detectable peak concentrations for levallorphan etorphine and morphine (3:1 signal-to-noise ratio) via fluorescence detection are *ca.* 5 pmoles per peak using our compromise emission wavelength and optimal separation. Naloxone is detectable with the same signal-to-noise ratio at 20 pmoles using UV absorbance at 225 nm. The lowered sensitivity in HPLC detection of levallorphan relative to its fluorescence efficiency is due to peak broadening and the compromise wavelength. However, these lower limits to detectability are essentially the same as those reported for electrochemical detection[4]. Our separation scheme is by no means the only one available for opiate alkaloids. However, we would like to emphasize the ionic strength dependence of separation efficiency which we found and which may be important in individual cases.

The reasons for the presence or absence of fluorescence for these compounds is an intriguing question since the compounds studied all possess a tyramine moiety whose UV absorbance at 280–290 nm gives rise to the fluorescence. Obviously, this is quenched in the case of naloxone and potentiated in levallorphan. The question is worth a theoretical investigation. However in practical terms the varying fluorescence yields may be of value since, for example, one may observe agonists (*e.g.*, morphine) in the presence of a much higher concentration of the antagonist naloxone.

ACKNOWLEDGEMENT

This work was supported by N.I.H. grant NS 15700, N.S.F. grant PCM 78-08543 and the University of Connecticut Research Foundation (J.A.G. principal investigator).

REFERENCES

1 K. Macek (Editor), *Pharmaceutical Applications of Thin-Layer and Paper Chromatography*, Elsevier, Amsterdam, London, New York, 1972, Ch. 15, pp. 444–447.
2 S. Udenfriend, *Fluorescence Assay in Biology and Medicine*, Vol. 2, Academic Press, New York, 1969, pp. 515–517.
3 I. Sunshine (Editor), *Handbook of Analytical Toxicology*, Chemical Rubber Publishing Co., Cleveland, OH, 1969.
4 M. W. White, *J. Chromatogr.*, 178 (1979) 229–240.
5 R. Gillilan, R. C. Lanman and W. E. Mason, *Anal. Lett.*, 13, No. B5 (1980) 381–387.