Journal of Chromatography, 381 (1986) 331—341
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3206

THIN-LAYER CHROMATOGRAPHIC AND COLUMN LIQUID CHROMATOGRAPHIC ANALYSES OF MORPHINE IN URINE VIA DABSYLATION

SHIH YUNG WANG* and SOCK YING THAM

School of Pharmaceutical Sciences, Sains Malaysia University, 11800 USM, Penang (Malaysia)

and

MIN KWONG POON

Pathology Laboratory, General Hospital, 10450 Penang (Malaysia)

(First received January 15th, 1986; revised manuscript received April 4th, 1986)

SUMMARY

A semiquantitative screening method for morphine in urine and a quantitative assay method for the drug were developed. In the semiquantitative method, morphine in urine was directly reacted with 4-dimethylaminoazobenzene-4′-sulphonyl chloride (dabsyl chloride) in a slightly alkaline medium. The orange-coloured dabsyl morphine was separated by silica gel thin-layer chromatography and the spot intensity was visually compared with that of the standards. The limit of detection is 0.075 μg/ml. In the quantitative method, morphine was extracted from urine before dabsylation. The dabsylation reaction is very fast and is complete within 5—10 min at room temperature. Dabsylation yield is maximum at a dabsyl chloride concentration of 6.2 m*M*. Total recovery of morphine using the extraction and dabsylation procedures described is 66%. Dabsyl morphine, thus formed, was analysed using high-performance liquid chromatography by monitoring its absorbance at 436 nm on a normal-phase μPorasil column. The limit of quantitation using high-performance liquid chromatography is 0.26 μ*M* (0.075 μg/ml), which corresponds to 10.5 pmol of injected dabsyl morphine. Quantitative assay was also carried out by thin-layer chromatography on silica gel followed by densitometry The limit of quantitation is 1.3 μ*M* (0.375 μg/ml).

INTRODUCTION

The various methods for morphine assay include thin-layer chromatography

0378-4347/86/$03.50

(TLC) [1—7], gas chromatography (GC) [8—10], high-performance liquid chromatography (HPLC) [6, 11—20], radioimmunoassay [21] and the enzyme multiplied immunoassay technique [22]. Among the chromatographic methods currently being used, TLC offers the advantage of simplicity of technique and low cost. Because of its lack of sensitivity and, to a certain extent, resolution, it is restricted to mainly qualitative and semiquantitative work. Thus, TLC is used widely for morphine screening in urine samples of drug addicts. Morphine, after separation by TLC, is usually detected by iodoplatinate spray [1—3]. However, it suffers from two disadvantages: the need for sample clean-up by solvent extraction and the variability of colour development. Moreover, low concentrations of morphine (< 0.2 μg/ml) are not detectable immediately after spraying. It is visible only after heating or storing at room temperature for several hours [1]. Detection of morphine on TLC by the fluorescence procedure has also been reported [4—7]. The use of fluorescence detection is sensitive down to nanogram levels of the drug. However, the biggest problem with this method is that the fluorescent derivative is sensitive to light.

For quantitative work, GC and HPLC are the methods of choice. The polar nature of morphine warrants pre-column or on-column derivatization for any meaningful quantitative estimation of the drug by GC. HPLC combines the merits of GC, i.e. precision, reliability, selectivity and efficiency, plus the advantage of operation at room temperature with no necessity for derivatization if the appropriate detector is used. Because of the moderate ultraviolet absorptivity of morphine, direct ultraviolet detection is not a very sensitive method [12]. Morphine can be converted to pseudomorphine, a fluorescent dimer, by reacting with alkaline potassium ferricyanide [13—15]. By using fluorescence detection, as little as 10 ng of morphine can be detected. Morphine can also be derivatized to a fluorescent product by reacting the drug with 1-dimethylaminonaphthalene-5-sulphonyl chloride (dansyl chloride), a fluorophore [6, 16]. Sensitivity can be increased to detect subnanogram levels of the drug. Besides, fluorescence detection can also be very selective. But light-sensitivity of dansyl morphine and quenching can pose serious problems in quantitative work. Electrochemical detection, which is very sensitive and requires no derivatization, is by far the best method for morphine detection [17—19]. However, not all laboratories are equipped with this type of detector.

4-Dimethylaminoazobenzene-4′-sulphonyl chloride (dabsyl chloride), first synthesized by Lin and Chang [23], has been increasingly used as a derivatizing agent for TLC and HPLC determinations of primary and secondary amines [24], amino acids [25, 26], putrescine, spermidine and spermine [27] and sphingosine [28]. Dabsylation has increased the sensitivity, with the detection limit down to the picomole level.

The present study explores the use of dabsylation for the semiquantitative screening and quantitative determination of morphine in urine. Morphine was treated with dabsyl chloride in a slightly alkaline medium to form dabsyl morphine, a chromophoric, orange product. This morphine derivative, unlike dansyl morphine, absorbs strongly in the visible region and is not light-sensitive. Semiquantitative screening was readily done by visual comparison of TLC spot intensities. Quantitative determinations were accomplished by measuring the absorbance of the HPLC effluent or the TLC spot at 436 nm.

EXPERIMENTAL

Materials

Unless stated otherwise, all solvents and reagents used were of analytical grade. Morphine sulphate pentahydrate (B.P. grade), which was obtained from the Government Medical Store (Petaling Jaya, Selangor, Malaysia), was used throughout the study. A stock solution of $2.63 \cdot 10^{-3}$ *M* (1 mg/ml) morphine sulphate pentahydrate in distilled water was prepared, from which various dilutions could be obtained.

All glassware was silanized using a 5% solution of dimethyldichlorosilane in toluene before use.

Preparation of dabsyl chloride

The method of Lin and Chang [23] was followed with slight modifications. A 4-g quantity of phosphorus pentachloride was thoroughly mixed with 2.5 g of sodium 4-dimethylaminoazobenzene-4′-sulphonate in an ice-cooled tall beaker for 5 min. The reaction mixture was poured onto 200 ml of an ice—water mixture; insoluble dabsyl chloride was collected by suction filtration and washed with water. The product was extracted with 150 ml of acetone at room temperature. On cooling down to −10°C, the acetone extract gave shiny, purple-red needles of dabsyl chloride with a yield of 50%.

If sodium 4-dimethylaminoazobenzene-4′-sulphonate was mixed with phosphorus pentachloride in a mortar according to the method of Lin and Chang [23], the reaction mixture first turned sticky and then formed a hard cake which made further mixing very difficult and usually resulted in poor yield.

Preparation of dabsyl morphine

Morphine sulphate pentahydrate (100 mg) in 10 ml of 1% sodium carbonate was mixed with 30 mg of dabsyl chloride in 10 ml of acetone. After standing at room temperature for 12 h, the reaction mixture was extracted with two 20-ml portions of benzene. The combined benzene extracts were washed with 40 ml of 0.1 *M* sodium hydroxide. The benzene layer, after drying over anhydrous sodium sulphate, was evaporated using a rotary evaporator to give 50 mg of residue. Recrystallization from benzene—acetone at 10°C yielded 30 mg of bright red plates, m.p. 215—217°C dec. The UV—VIS spectrum was recorded on a Pye Unicam SP7-500 UV—VIS spectrophotometer.

Enzyme hydrolysis of urine

The pH of the urine sample was measured by using pH paper and adjusted to 7 by adding acetic acid if found to be basic. Then, 0.1 ml of 0.1 *M* sodium acetate—acetic acid buffer (pH 5.5) and 0.02 ml of β-glucuronidase solution (75 U/ml) were added to each ml of urine. The urine sample was incubated at 40—45°C for 18 h.

Semiquantitative TLC screening

To 1.0 ml of enzyme-hydrolysed urine in a 5-ml Reacti vial (Pierce, Rockford, IL, U.S.A.), 0.5 ml of 1 *M* sodium bicarbonate and 1 ml of dabsyl chloride solution (2 mg/ml in acetone) were added. The Reacti vial was closed

tightly and shaken to mix the solutions. Red solids were formed, presumably from the precipitation of dabsyl chloride. The Reacti vial was heated in a water bath at 70°C until the solids dissolved (for ca. 10 min). On cooling down to room temperature the reaction mixture was treated with 0.5 ml of 5 *M* sodium hydroxide to raise the pH and it was then extracted with 100 μl of toluene—heptane (1:1) mixture. The lower aqueous phase was thrown away and the upper organic phase was further washed with 1 ml of 0.5 *M* sodium hydroxide. TLC analysis was carried out on a silica gel 60 F_{254} precoated plate, layer thickness 0.25 mm (Merck, Darmstadt, F.R.G.). The height of the plate was always 5 cm, the width, however, depended on the number of samples. Toluene—heptane extract (5 μl) was applied onto the TLC plate with a 5-μl Microcap pipette (Drummond Scientific, Broomal, PA, U.S.A.) in four portions so that the spot diameter was < 4 mm. In between applications, the spot was dried with a hair-dryer. Comparison standards made from spiked normal urine (0.225, 0.75, 2.25 and 7.5 μg/ml) were applied onto the same plate. Spots were applied at a constant distance of 4 mm apart and 1.0 cm from the bottom.

The glass TLC developing chamber (9 × 3 × 7 cm) was lined with filter paper and filled with the developing solvent, chloroform—absolute ethanol—triethylamine (30:2:0.05), to a height of 0.5 cm. The plate was developed until the solvent front reached the top edge after ca. 5—7 min. After removal, the plate was dried in a stream of warm air for 1 min and then observed under daylight.

Quantitative determination using HPLC

Aliquots of 2 ml of the enzyme-hydrolysed blank urine were spiked with morphine sulphate pentahydrate at urine morphine concentrations of 0.26, 1.32, 2.64, 7.92 and 15.84 μ*M*. Spiked urine samples and urine blank were saturated with sodium chloride. A 300-μl volume of 1 *M* sodium bicarbonate—sodium hydroxide buffer (pH 9.1) was added to each urine sample to ensure a urine pH of 8—9. Each urine sample was then extracted with 2 ml of a chloroform—isopropanol (9:1) mixture. A 0.5-ml aliquot of the organic layer was transferred to a 1-ml Reacti vial and the chloroform—isopropanol extract evaporated to dryness in a stream of nitrogen.

A 50-μl volume of 12.4 m*M* dabsyl chloride in acetone and 50 μl of 0.1 *M* sodium carbonate were added into each dried urine extract. The mixture was shaken vigorously and allowed to stand at room temperature for 15 min. On completion of dabsylation, the reaction mixture was treated with 50 μl of 5 *M* sodium hydroxide followed by extraction into 250 μl of toluene. The toluene extract was ready for HPLC and TLC quantitation.

The HPLC instrument consisted of a Gilson Model HM/Holochrome UV monitor with a deuterium light source set at 436 nm, a Gilson Model 302 pump, a Gilson Model 802 manometric module, a Rheodyne Model 7125 injector and a Shimadzu C-R1B Chromatopac integrator—plotter. A 20-μl aliquot of the toluene extract was injected using the complete loop filling method. The technique of syringe rinsing was standardized to five rinses with toluene followed by four rinses with the solution to be injected. With repeated injections of the same solution, the coefficient of variation was < 3% (n = 10). The dabsylated extracts were separated on a normal-phase Waters μPorasil

(fully porous silica gel, particle size 10 μm) 30 cm × 3.9 mm I.D. column using chloroform—95% ethanol (20:2) containing 1 drop of triethylamine per 100 ml of mixture as the mobile phase at a flow-rate of 1 ml/min.

Quantitative determination using TLC

For TLC quantitation, 5 μl of the toluene extract were chromatographed on a silica gel plate in a manner identical to that described in the semiquantitative screening procedure. To suit the x parameter of the scanner, the spots were 6 mm apart. The developed plate was scanned at 436 nm on a Shimadzu CS-920 high-speed TLC scanner with the following parameters: x = 6 mm, y = 10 mm, z = 6 mm, Lz = 1, AZS = off.

RESULTS AND DISCUSSION

Since morphine is excreted mostly as its glucuronide conjugate, we hydrolysed all our urine samples before chemical analysis. Enzyme hydrolysis was found to give a much cleaner background on the thin-layer chromatogram compared with acid hydrolysis. Subsequent pH adjustments were also easier in the case of enzyme hydrolysis.

With the aim of developing a simple and rapid screening method, we dabsylated the whole urine, without any preliminary solvent extraction. Following standard dabsylation procedures [24, 28], the reaction was carried out in an acetone—water mixture in the presence of sodium bicarbonate. Because of the presence of endogenous ammonia, amines and amino acids in the urine, a large excess of dabsyl chloride was used. At the end of the reaction, the reaction mixture was made strongly alkaline by the addition of sodium hydroxide and then extracted with an organic solvent. Due to their acidic nature, dabsyl derivatives of ammonia, primary amines and amino acids should stay in the strongly alkaline aqueous phase. Fig. 1 compares the chromatograms of a spiked urine sample where the extractions were carried out with or without the addition of sodium hydroxide. High pH in the aqueous phase decreases the interferences by endogenous substances drastically but the intensity of dabsyl morphine spot is not affected. The extraction solvent heptane—toluene

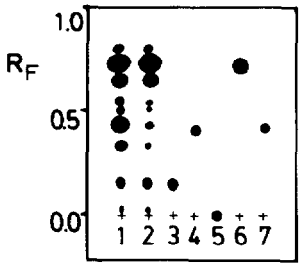


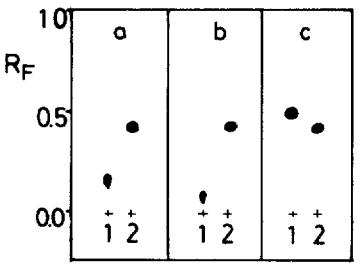
Fig. 1. Thin-layer chromatogram of dabsyl morphine and related substances on silica gel 60 F_{254} in chloroform—absolute ethanol—triethylamine (30:2:0.05). Samples: (1) morphine in urine (3 μg/ml), dabsylated and extracted from sodium bicarbonate medium; (2) morphine in urine (3 μg/ml), dabsylated and extracted from sodium hydroxide medium; (3) dabsyl morphine; (4) dabsyl nalorphine; (5) sodium 4-dimethylaminoazobenzene-4′-sulphonate; (6) dabsyl chloride; (7) dabsylamide.

mixture is a good compromise between its extracting efficiency and its eluting power. It is strong enough to effect an essentially complete extraction of dabsyl morphine from the aqueous phase, yet it is weak enough not to cause substantial spreading of dabsyl morphine during sample application onto TLC. Although the sample spot diameter can be as big as 4 mm, the dabsyl morphine spot is always < 2 mm in diameter on the developed chromatogram. This results in excellent resolution and high sensitivty of the method.

In preliminary experiments, a solvent system of chloroform—absolute ethanol (30:2) was used to develop the TLC plate. It separated dabsyl morphine very well from sodium 4-dimethylaminoazobenzene-4′-sulphonate, dabsyl chloride, dabsylamide and dabsyl derivatives of other contaminants in the urine. However, it was found that accidental exposure of the TLC plate to acetic acid vapour lowers the R_F value and causes slight tailing of the dabsyl morphine spot. Exposure of TLC to ammonia vapour results in a larger R_F value (Fig. 2). The observed dependence on the pH of the stationary phase is presumably caused by the presence of a tertiary amino group in the morphine moiety. At lower pH, dabsyl morphine is protonated and thus shows greater affinity for the silica gel. This explanation is further augmented by the observation of the lack of pH influence on the TLC behaviour of dabsylamide, a molecule without the amino group. To avoid the possible exposure to acidic or basic substances in the atmosphere, all the TLC plates were stored in a closed container until use once the manufacturer's packaging was opened.

With the aim of suppressing the ionization of dabsyl morphine, we added a slight amount of triethylamine to the eluting solvent. As can be seen in Fig. 3, the R_F values were not much affected but the spots were more circular.

The slight concentration gradient in the mobile phase along the direction of development made the R_F value of dabsyl morphine slightly dependent on the height of the eluting solvent in the solvent tank. Therefore, a fixed height of 0.5 cm of solvent was used and the development was stopped at 4 cm from the origin. Under these conditions, a consistent R_F value of 0.17 was obtained. The detection limit is 7.5 ng of dabsyl morphine, corresponding to a urine



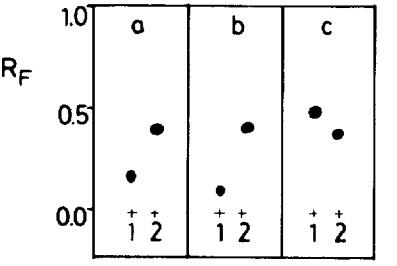


Fig. 2. Effect of pH of the stationary phase on the TLC behaviour of dabsyl morphine and dabsylamide in chloroform—absolute ethanol (30:2). (a) Silica gel 60 F_{254}; (b) silica gel 60 F_{254} exposed to acetic acid vapour; (c) silica gel 60 F_{254} exposed to ammonia vapour. Samples: (1) dabsyl morphine; (2) dabsylamide.

Fig. 3. Effect of pH of the stationary phase on the TLC behaviour of dabsyl morphine and dabsylamide in chloroform—absolute ethanol—triethylamine (30:2:0.05). Notations are as in Fig. 2.

morphine concentration of 0.075 μg/ml if 1 ml of urine was used in the analysis. The whole screening process takes less than 2 h for ten samples.

To check the possible interferences from a few other drugs that may be used by drug addicts, we treated codeine, amphetamine, methamphetamine, cocaine, nicotine and phenobarbitone with the same dabsylation and TLC procedures. They either do not react with dabsyl chloride or give a derivative that moves close to the solvent front. Nalorphine, a morphine antagonist, gave an R_F value of 0.43.

Twenty urine samples of heroin-abuse suspects were screened by this method. Visual comparison of intensities of spots gave the semiquantitative results in Table I. The results agree quite well with those determined following the TLC—iodoplatinate method [2] and the GC method [9]. On the basis of these results, it was concluded that TLC following dabsylation provides a rapid semiquantitative screening method for morphine in urine.

In extending the dabsylation method to HPLC quantitation of morphine, we monitored the absorbance of the HPLC effluent at 436 nm, a wavelength readily available on either the variable-wavelength detector or the fixed-wavelength detector. A comparison of the molar absorptivities of dabsyl morphine and morphine shows that in methanol solution, the molar absorptivitiy of dabsyl morphine at its absorption maximum of 450 nm ($\epsilon = 3.5 \cdot 10^4$) is about thirty times stronger than that of morphine at its absorption maximum of 280 nm ($\epsilon = 1.4 \cdot 10^3$). This promises a high sensitivity for the dabsylation method.

TABLE I

COMPARISON OF RESULTS OF URINE MORPHINE ANALYSES

No.	TLC—iodoplatinate*	Semiquantitative TLC screening (μg/ml)	GC** (μg/ml)
1	±	<0.225	0.13
2	—	—	0.15
3	±	<0.225	0.15
4	—	<0.225	0.24
5	+	ca. 0.225	0.38
6	+	ca. 0.75	1.28
7	+	ca. 0.75—2.25	1.29
8	+	ca. 2.25	2.46
9	+	ca. 2.25	3.73
10	+	ca. 2.25—7.5	3.98
11	+	ca. 2.25—7.5	4.22
12	+	ca. 2.25—7.5	8.55
13	+	>7.5	9.76
14	+	>7.5	12.11
15	+	>7.5	17.63
16	+	>7.5	25.76
17	+	>7.5	28.41
18	+	>7.5	31.10
19	+	>>7.5	84.17
20	+	>>7.5	109.30

*Following the method of Lopez et al. [2]. —, Negative; +, positive; ±, weak positive.
**Following the method of Dutt et al. [9].

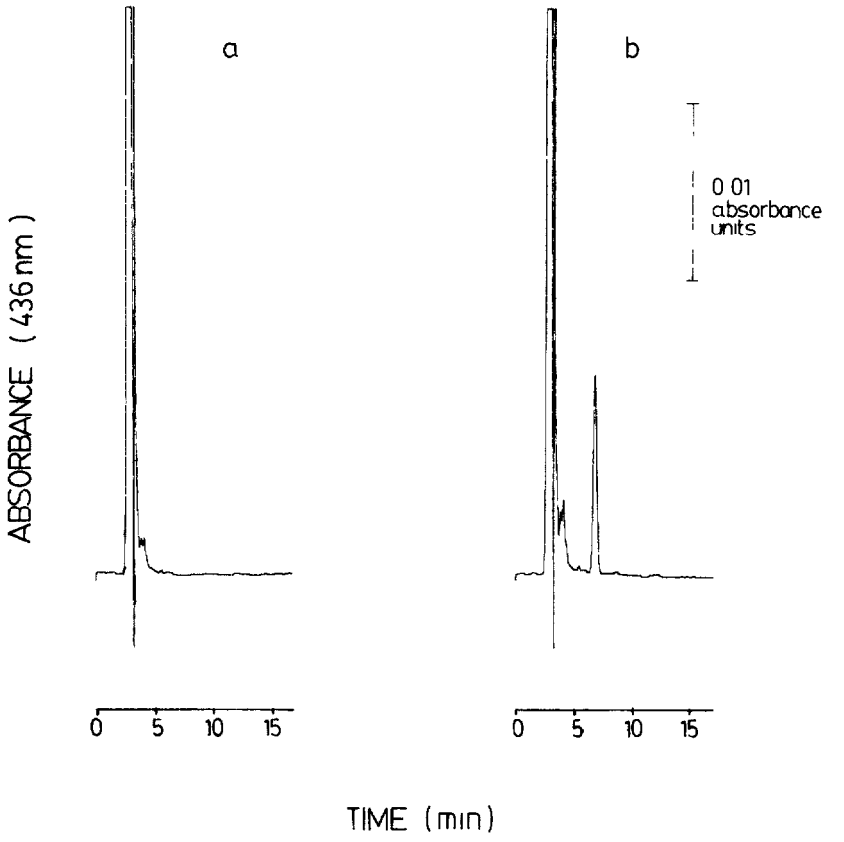


Fig. 4. HPLC profiles of (a) urine blank and (b) urine sample spiked with 7.92 μM morphine, which were extracted and dabsylated as described in text. Chromatographic conditions: column, μPorasil (30 cm × 3.9 mm I.D.); mobile phase, chloroform—95% ethanol (20:2) with 1 drop of triethylamine per 100 ml of mixture; flow-rate, 1 ml/min; detector, 436 nm.

Normal-phase HPLC was found to be more suitable for the analysis of dabsyl morphine. Satisfactory separation of dabsyl morphine with a retention time of 7 ± 0.1 min was achieved isocratically by using the mobile phase of chloroform—95% ethanol (20:2) containing a drop of triethylamine per 100 ml of mobile phase (Fig. 4). The composition of the mobile phase was adapted from the TLC developing solvent. Triethylamine was added to the mobile phase to suppress the ionization of dabsyl morphine and to obtain a more consistent retention time and a more symmetrical peak shape for dabsyl morphine. In the absence of triethylamine in the mobile phase, the retention time of dabsyl morphine fluctuated between 6 and 10 min. The water—alcohol mixture (5:95) is essential to deactivate the silica gel stationary phase. When chloroform—absolute ethanol (20:2) was used as the mobile phase, dabsyl morphine could not be eluted out even after 1 h. The urine blank sample shows a clean chromatogram, particularly near the dabsyl morphine peak. As can be seen in Fig. 4a, endogenous urine contaminants are eluted close to the solvent peak and do not interfere with the dabsyl morphine peak.

With quantitative determination of morphine using HPLC, it is imperative to

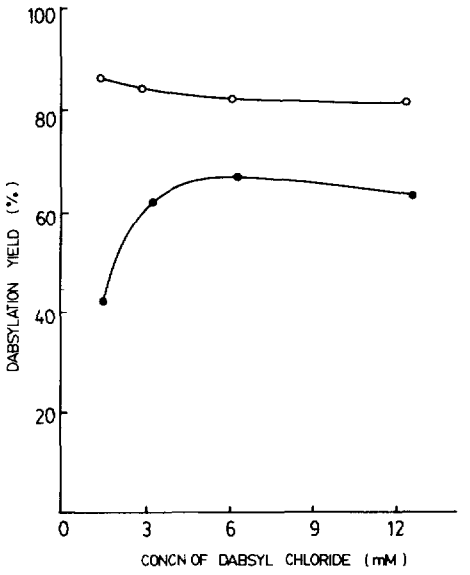


Fig. 5. Effect of dabsyl chloride concentration on morphine dabsylation; 7.92 nmol of pure morphine (○) and 7.92 nmol of morphine extracted from 0.5 ml of spiked urine (●) were dabsylated at various reagent concentrations. Chromatographic conditions are as in Fig. 4.

perform a preliminary extraction of the drug from urine because inconsistent peak-area values of dabsyl morphine were obtained when dabsylation was performed directly on spiked whole urine samples. The work on oestrogens demonstrated that water content in the reaction medium plays an important role in the reaction of sulphonyl chloride with phenolic groups [29, 30]. Higher water content increases the rate of formation as well as the rate of hydrolysis of the sulphonate. The net result is a faster drop in sulphonation yield with time. Hence, in our investigation the ratio of water to acetone in the reaction mixture was kept as low as possible, to slow down the hydrolysis of dabsyl morphine.

In the present study we investigated the effect of dabsyl chloride reagent concentration on the extent of dabsylation of morphine (Fig. 5). When 7.92 nmol (2.26 μg) of pure morphine were dabsylated in 100 μl of acetone—water (1:1) medium containing 0.05 *M* sodium carbonate and varying dabsyl chloride concentration from 1.55 to 12.4 m*M*, dabsylation yield was constant. Based on the peak area of authentic dabsyl morphine, dabsylation yield was estimated to be around 83%. However, when the residue from the extraction of 0.5 ml of normal urine spiked with 7.92 nmol of pure morphine was dabsylated under the same conditions, the dabsylation yield increased with an increase in dabsyl chloride concentration from 1.55 to 6.2 m*M*. A further increase of dabsyl chloride concentration to 12.4 m*M* resulted in a slight decrease in the dabsylation yield. Even at the optimal concentration of 6.2 m*M* dabsyl chloride, dabsylation yield was only 66%, about 80% of the dabsylation yield of pure morphine. This difference in dabsylation yield may be attributed to the loss of morphine during the chloroform—isopropanol extraction. This reagent concentration dependence is in agreement with what has been reported by previous

workers on the dabsylation of amino acids and sphingosine [25, 28].

We found that at 6.2 m*M* dabsyl chloride and 0.05 *M* sodium carbonate in a 1:1 acetone—water medium, dabsylation of morphine, extracted from urine, achieved its maximum after 5—10 min. Under these conditions there was no appreciable drop in dabsylation yield with reaction time up to 90 min, indicating that there was no appreciable amount of hydrolysis of dabsyl morphine.

The orange-coloured dabsyl morphine in toluene extract was found to be stable for up to two to three months at room temperature. Hence, no precautions were necessary to store this morphine derivative in the dark.

Prior to chloroform—isopropanol extraction of morphine from urine, saturation of the aqueous phase with sodium chloride was performed. This increases the recovery of morphine, at a concentration of 15.84 μM, from 67 to 80%. Apart from this, silanizing glassware is essential to overcome adsorption of morphine to the surface of the glassware. The recovery of morphine has been reported to improve by approximately 20% by using siliconized glassware [13].

The linear calibration curve, with a correlation coefficient of 1.0000, for urine morphine concentrations ranging from 0.26 to 15.84 μM passes through the origin. The limit of quantitation for morphine in urine is 0.26 μM (0.075 μg/ml), based on a signal-to-noise ratio of 3. This concentration corresponds to 10.5 pmol of injected dabsyl morphine. When precision was assessed on spiked urine samples containing 15.84 μM morphine, the coefficient of variation was 5.7% ($n = 6$).

For quantitative assay by TLC, a modern high-speed zig-zag scanner was used. In-situ reflectance measurements yielded area readings proportional to the dabsyl morphine content in the range 1.3—15.8 μM, with a correlation coefficient of 0.9990. All samples were chromatographed on the same TLC plate in order to minimize the variation in chromatographic conditions. Coefficients of variation for the repeated assays of spiked urine samples were around 5—7% in the range studied. The source of variation comes mainly from the sample preparation and the spotting step. Variation in repeated densitometric measurements of single spotting is very small (< 1%) and hence considered negligible. The limit of quantitation is 1.3 μM (0.375 μg/ml).

Comparison of HPLC and TLC results indicates that the coefficients of variation of the two methods are comparable but the limit of quantitation of TLC is higher than that of HPLC. This is caused mainly by the limitation of sample application of the TLC method. Application of larger amount of sample solution to a TLC plate is time-consuming and results in a large spot diameter. Nevertheless, TLC also has a few advantages over HPLC: several samples can be analysed simultaneously; there is no problem of column degradation as in HPLC, therefore sample clean-up is not as stringent; a wider choice of eluting solvents can be used. Furthermore, the TLC method consumes less solvent and hence the cost per sample is much lower than for the HPLC method.

ACKNOWLEDGEMENTS

The authors thank the National Drug Research Centre for providing the facilities for the densitometry work. This study was funded by a short-term

research grant (Grant No. 280202) from the Sains Malaysia University, Penang, Malaysia.

REFERENCES

1 J.E. Wallace, J.D. Biggs, J.H. Merritt, H.E. Hamilton and K. Blum, J. Chromatogr., 71 (1972) 135.
2 J.B. Lopez, J.E. Buttery and G.F. de Witt, Mod. Med. Asia, 14 (1978) 7.
3 K.K. Kaistha and R. Tadrus, J. Chromatogr., 267 (1983) 109.
4 H.H. Loh, I.K. Ho, T.M. Cho and W. Lipscomb, J. Chromatogr., 76 (1973) 505.
5 J. Sherma, M.F. Dobbins and J.C. Touchstone, J. Chromatogr. Sci., 12 (1974) 300
6 R.W. Frei, W. Santi and M. Thomas, J. Chromatogr., 116 (1976) 365.
7 R. Wintersteiger, Analyst, 107 (1982) 459
8 P.O. Edlund, J. Chromatogr., 206 (1981) 109.
9 M.C. Dutt, D.S.-T. Lo, D.L.K. Ng and S.-O. Woo, J. Chromatogr., 267 (1983) 117.
10 K.E. Rasmussen, J. Chromatogr., 120 (1976) 491.
11 C. Olieman, L. Maat, K. Waliszewski and H.C. Beyerman, J. Chromatogr., 133 (1977) 382.
12 J.-O. Svensson, A. Rane, J. Säwe and F. Sjöqvist, J. Chromatogr., 230 (1982) 427.
13 A.E. Takemori, Biochem. Pharmacol., 17 (1968) 1627.
14 I. Jane and J.F. Taylor, J. Chromatogr., 109 (1975) 37.
15 P.E. Nelson, S.L. Nolan and K.R. Bedford, J. Chromatogr., 234 (1982) 407.
16 F. Tagliaro, R. Dorizzi, M. Plescia, M. Pradella, S. Ferrari and V. Lo Cascio, Fresenius' Z. Anal. Chem., 317 (1984) 678.
17 M.W. White, J. Chromatogr., 178 (1979) 229.
18 R.B. Raffa, J.J. O'Neill and R.J. Tallarida, J. Chromatogr., 238 (1982) 515.
19 J.A. Owen and D.S. Sitar, J. Chromatogr., 276 (1983) 202.
20 M. Arunyanart and L.J. Cline Love, J. Chromatogr., 342 (1985) 293.
21 S. Spector, J. Pharmacol. Exp. Ther., 178 (1971) 253.
22 R.S. Schneider, P. Lindquist, E.T.I. Wong, K.E. Rubenstein and E.F. Ullman, Clin. Chem., 19 (1973) 821.
23 J.K. Lin and J.Y. Chang, Anal. Chem., 47 (1975) 1634.
24 J.K. Lin and C.C. Lai, Anal. Chem., 52 (1980) 630.
25 J.Y. Chang, R. Knecht and D.G. Braun, Biochem. J., 199 (1981) 547.
26 J.Y. Chang, R. Knecht and D.G. Braun, Biochem. J., 203 (1982) 803.
27 J.-K. Lin and C.-C. Lai, J. Chromatogr., 227 (1982) 369.
28 G.Rosenfelder, J.-Y. Chang and D.G. Braun, J. Chromatogr., 272 (1983) 21.
29 G.W. Oertel and L.P. Penzes, Z. Anal. Chem., 252 (1970) 306.
30 L.P. Penzes and G.W. Oertel, J. Chromatogr., 51 (1970) 325.
31 J.A. Vinson and A.H. Patel, J. Chromatogr., 307 (1984) 493.