

## Discussion

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# High-performance liquid chromatographic determination of morphine, morphine-3-glucuronide, morphine-6-glucuronide and codeine in biological samples using multi-wavelength forward optical detection

## Reply to Bogusz

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We thank Dr. Bogusz for his comments regarding the above-mentioned paper [1]. He has raised some pertinent points which were not mentioned in our paper.

The sensitivity which was obtained in this study could only be achieved by using the data manipulation capabilities of the UV-VIS Spectra Focus forward optical detector and, more importantly, the Spectra Focus software (Spectra-Physics, San Jose, CA, USA). This program has the ability to subtract and ratio chromatograms and spectra. This process is very tedious and time-consuming. Without this capability, the spectral data were definitely distorted at lower detection limits. Incidentally, this was not detailed in our paper.

In response to Dr. Bogusz's other comments,

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first, the three-dimensional chromatogram (Fig. 1) is a result of ratioing and subtraction between two chromatograms, one with morphine, its metabolites and several interfering peaks and the other without morphine or its metabolites but with the interfering peaks. The absorbance units shown in Fig. 1 which led to the comments by Dr. Bogusz do not represent real units. We regret the misunderstanding which has arisen.

The chromatograms shown in Fig. 2 were again obtained after ratioing and subtraction as mentioned above. We agree that Fig. 3 (chromatogram of extracted blank plasma) represents a particularly "dirty extract".

Figs. 4 and 5 showing chromatograms of cerebrospinal fluid (CSF), plasma and urine samples of an infant obtained 24 h after the administration of morphine were the result of subtraction and ratioing. The concentrations of morphine (M), morphine-3-glucuronide (M-3-G) and morphine-6-glucuronide (M-6-G) given in his com-

TABLE I

CONCENTRATIONS OF MORPHINE (M), MORPHINE-3-GLUCURONIDE (M-3-G) AND MORPHINE-6-GLUCURONIDE (M-6-G) IN TWO INFANTS FOUND 24 h AFTER SINGLE INTRAVENOUS ADMINISTRATION OF MORPHINE

Infant No.	Sample	Concentration (ng/ml)		
		M	M-3-G	M-6-G
1	CSF	Not done	Not done	Not done
	Plasma	3.5	3.7	2.1
	Urine	19.2	10.2	3.2
2	CSF	Not done	Not done	Not done
	Plasma	5.5	5.7	2.2
	Urine	12.3	9.6	4.6

ments are not correct because of the confusion over the absorbance units. The actual values we obtained are given in Table I.

Both infants were premature and acutely ill. The morphine levels shown above were similar to the levels detected by radioimmunoassay [2]. We are not claiming that this is a particularly easy technique, but by using the program capabilities it is possible to obtain chromatograms that are

not distorted and to detect comparatively lower levels.

#### REFERENCES

- 1 G. Chari, A. Gulati R. Bhat and I. R. Tebbett, *J. Chromatogr.*, 571 (1991) 263.
- 2 R. Bhat, G. Chari, A. Gulati, O. Aldana, R. Velamati and H. Bhargava, *J. Pediatr.*, 117 (1990) 477.