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*Short Communication*

# Application of photodiode array UV detection in the development of stability-indicating LC methods: determination of mefenamic acid

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## Introduction

Analytical methods must be validated so that the performance characteristics meet the requirements for the intended analytical application. Many excellent papers on validation of analytical methods have been published [1–7], and typical analytical parameters used in assay validation have been specified [1, 2, 4, 7–10]: these include precision, accuracy, limit of detection, limit of quantitation, selectivity, linearity and ruggedness. In particular, development of a stability-indicating assay to monitor unchanged drug is of paramount importance in the course of stability studies on pharmaceutical dosage forms [11–14].

The selectivity (specificity) of an analytical method determines its ability to measure accurately and precisely the analyte in the presence of components of the sample matrix such as inactive ingredients, impurities and degradation products [15, 16]. In the development of a stability-indicating assay, selectivity is the most critical criterion of the method's validity.

Liquid chromatography (LC) is usually the analytical technique most suitable for a stability-indicating assay of pharmaceuticals. Although LC has powerful resolving capacity, it is still quite often a challenge for the analytical chemist to develop a truly specific assay method.

It is usually not difficult to check for interferences from the placebo ingredients or impurities. A placebo mixture can be easily prepared and analysed. Information on inherent impurities is often available from the drug manufacturer, as are authentic samples of relevant chemicals. The most difficult problem that the analyst faces is validation of the method's selectivity against potential degradation products. Even for commonly used drugs, potential degradation products are not always known, and reference chemicals are frequently not available. In many cases, this problem can be overcome by producing degradation products, *in situ*, under different stress conditions, and analysing them without isolation or identification. Often degradation products are chromatographically very similar to the parent compound and they may elute at the same time. Therefore, it is critical to evaluate peak identity and homogeneity: whether the eluting peak observed on a chromatogram represents one component or a coeluting mixture of two or more components. Therefore, a technique that permits judgement with some certainty of the purity of an eluting chromatographic peak is of particular benefit to the analytical chemist.

There are several methods of determining the purity of a peak, including techniques such as comparing the retention time of the eluting peak with the retention time of a known

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standard, comparative detection techniques (e.g. UV versus electrochemical), spectral overlays, absorbance ratios, and most recently photodiode array detection. The advantages and deficiencies of these techniques have been reviewed by Tompkins [17] and Alfredson and Sheehan [18].

Photodiode array detection with numerical output format (purity parameter), is very useful in assessing peak identity and integrity. Objectivity is the main advantage of the numerical schemes and the evaluation of the peak's purity is straightforward. Compared with most graphical diode array outputs, the numerical format also allows the data to be reduced more rapidly.

The purity parameter employs an absorbance weighting factor in order to minimize the effect of noise on the calculations. This format yields a single value in units of nanometers that represents a characteristic average wavelength (weighted mean wavelength) of a spectrum. The calculation of purity parameter is analogous to a statistical calculation of the moment of a distribution. The wavelength range over which the purity parameter is calculated can be selected to focus on a characteristic absorbance band or bands of a spectrum to enhance discrimination between the spectra of two different compounds, thereby increasing the specificity of the method.

## Experimental

*Reagents*

Mefenamic acid (Parke-Davis, Holland, Michigan), glacial acetic acid and 0.5 N sodium hydroxide (BDH, Toronto, Ontario) were used as received. All solvents were HPLC grade (Caledon, Georgetown, Ontario).

*Equipment*

The HPLC system consisted of a model 6000A pump, a WISP 710-B autosampler (both Waters Associates, Mississauga, Ontario), a Polychrom 9060 photodiode array detector (Varian, Georgetown, Ontario), a Hewlett Packard 3390 integrator and a Think Jet printer (Hewlett Packard, Mississauga, Ontario).

*Chromatographic conditions* I

A reversed-phase 10 μm μBondapak Phenyl Column (10 μm, 300 × 3.9 mm) was used, together with a Guard-Pak Precolumn Module and a μBondapak C18 disposable insert (all Waters Associates). The analytical wavelength for peak detection was set at 278 nm on a Polychrom 9060 detector. Mobile phase was methanol–glacial acetic acid–water (85:2:15, v/v). The flow rate was set at 1 ml $min^{-1}$ and the resultant pressure was approximately 2800 psi.

*Chromatographic conditions* II

An HPLC method previously developed in Parke-Davis laboratories (McLaughlin, unpublished data) was modified as follows.

A reversed-phase Nova-Pak C18 column (5 μm, 150 × 3.9 mm; Waters) and the precolumn system described under Chromatographic conditions I were employed. The mobile phase composition was acetonitrile–THF–water–glacial acetic acid (15:40:45:2, v/v). The flow rate was set at 1 ml $min^{-1}$ and the resultant pressure was approximately 1500 psi. All the other parameters were set as described in Chromatographic conditions I.

*Preparation solvent*

The sample preparation solvent was obtained by adding 20 ml of glacial acetic acid to 1 l of methanol.

*Standard preparation*

A standard solution of mefenamic acid was prepared in the preparation solvent to contain 5 μg $ml^{-1}$.

In situ *preparation of degradation products*

Degradation products of mefenamic acid were prepared, *in situ*, as specified below:

*Sample* 1. *Decomposition under acidic conditions*. Mefenamic acid (94.5 mg) was refluxed with 20 ml of 0.5 N $H_2SO_4$ for 48 h.

*Sample* 2. *Decomposition under basic conditions*. Mefenamic acid (81.6 mg) was refluxed with 20 ml of 0.1 N NaOH for 48 h.

*Sample* 3. *Decomposition under oxidative conditions*. Mefenamic acid (107.0 mg) was refluxed with 30 ml of 30% $H_2O_2$ and 1 ml 1 N HCl for 24 h.

After the specified times, the reaction mixtures were quantitatively transferred to separate 200 ml volumetric flasks, and adjusted to volume with methanol (sufficient DMSO was

added if a precipitate insoluble in MeOH was present).

## Results and Discussion

*Analysis of the degraded samples*

The concentrated solutions of samples 1–3 (about 5 μg $ml^{-1}$) were filtered and chromatographed for 1 h each under Chromatographic conditions I. The respective chromatograms, together with the chromatogram of the mefenamic acid standard solution are presented in Fig. 1.

The chromatogram of sample 1 (acidic degradation) showed only one peak with a retention time of approximately 4 min (Fig. 1a). Sample 2 showed some additional minor peaks at approximately 5.6 and 7 min (Fig. 1b). Chromatogram of sample 3 was the most complex and indicated extensive decom-

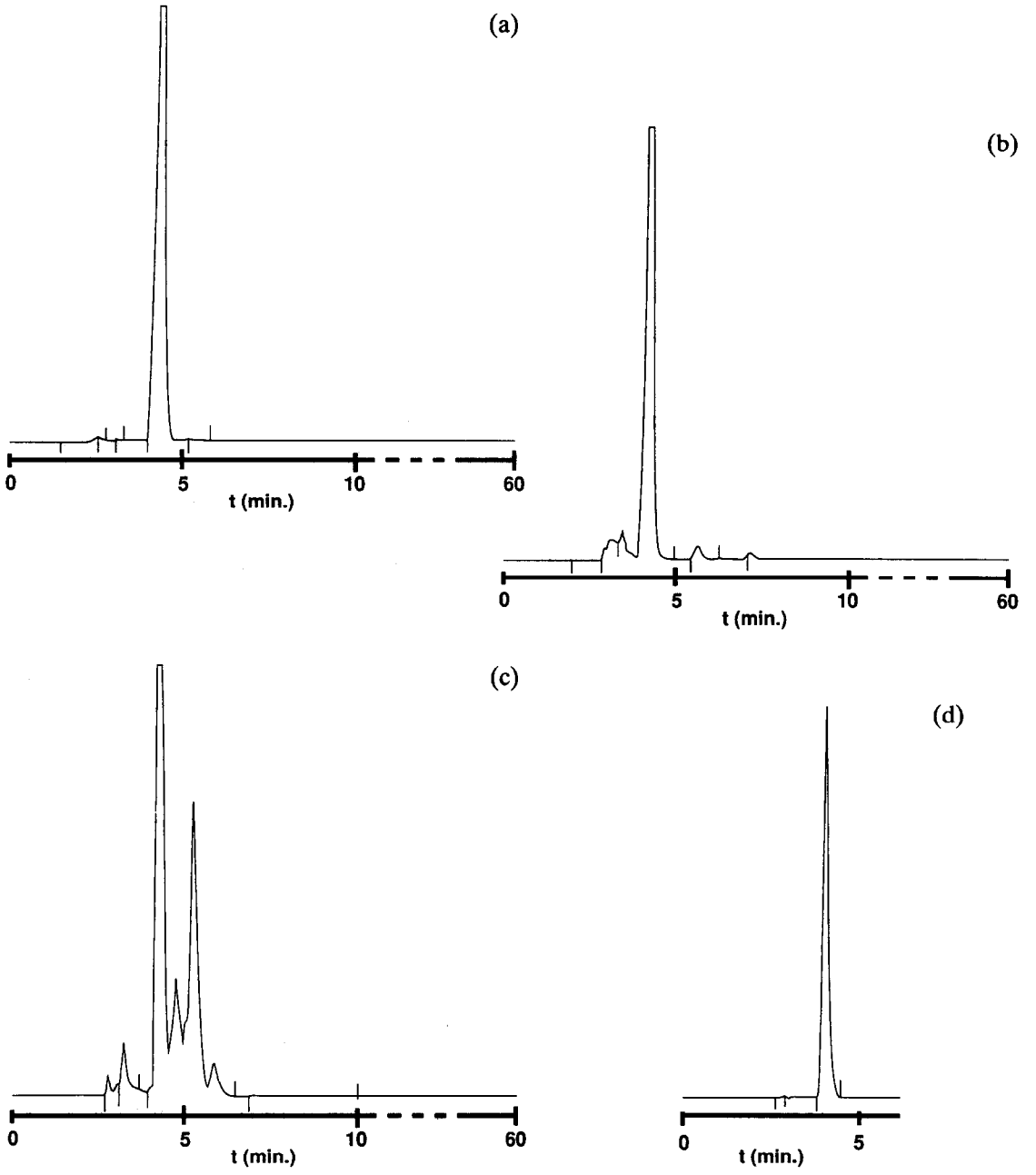


**Figure 1**
Chromatograms of mefenamic acid and its degradation products, *in situ*, under (a) acidic, (b) basic, and (c) oxidizing conditions. Chromatogram (d) represents a mefenamic acid standard.

position of mefenamic acid (Fig. 1c). All three samples after dilution with methanol, (1:10) were assayed for unchanged mefenamic acid using an external standard method and Chromatographic conditions I. The recoveries were: sample 1, 98.5%; sample 2, 102.6%; and sample 3, 63.8%.

The next step in the analysis of the forced degradation products involved peak identity and homogeneity checks employing the purity parameter under Chromatographic conditions I and the photodiode array detector set at the wavelength range 249–367 nm. Purity parameters obtained for samples 1–3 and for the mefenamic acid standard are listed in Table 1.

From the analysis of purity parameters, it was concluded that unchanged mefenamic acid appeared as a pure peak, resolved from degradation peaks in samples 2 and 3. In contrast, in sample 1, the peak at about 4 min clearly represented mefenamic acid coeluting with another compound or compounds. Therefore, it was necessary to modify the chromatographic conditions in such a way that the mefenamic acid peak was separated from the coeluting peak or peaks. Chromatographic conditions II resolved mefenamic acid and the product of acidic hydrolysis (Fig. 2). The degradation product was identified as *N*-phenyl-2,3-xylidine by comparison of the retention times, UV spectra, and purity parameters of the unknown and pure *N*-phenyl-2,3-xylidine.

All reaction mixtures obtained during forced degradation experiments were re-examined under the new chromatographic conditions. Their chromatograms and purity parameters for the mefenamic acid peak were compared with the corresponding data for the mefenamic acid standard. In all samples, the unchanged mefenamic acid was well resolved from degradation products. Therefore, the LC method based on Chromatographic conditions II was considered to be stability-indicating.

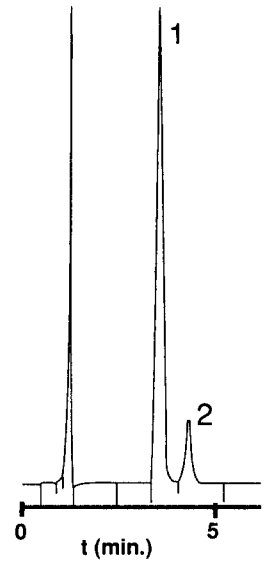


**Figure 2**
Chromatogram of the acidic degradation products of mefenamic acid (Chromatographic conditions II): 1, mefenamic acid; 2, degradation product.

*Validations*

*Placebo sample.* A placebo sample was prepared and analysed under Chromatographic conditions II. No interference from the placebo ingredients was observed.

*Linearity.* Detector response (peak height) was linear for the mefenamic acid concentration range between 25–150 μg $ml^{-1}$ (50–300% of the expected sample concentration). The straight line passed through the origin and the correlation coefficient ($r$) was 0.9993 for $n = 4$.

*Reproducibility.* Six consecutive injections of the standard preparation gave an RSD of 0.4%.

*Accuracy.* Six placebo samples were spiked with mefenamic acid at 100% of the label

**Table 1**
Purity parameters for mefenamic acid peak in the standard solution and three degradation mixtures (detector range 249–367 nm)

| Time (min) | Peak type | Standard | Purity parameters Sample 1 $[H^+]$ degr. | Sample 2 $[OH^-]$ degr. | Sample 3 [O] degr. |
|---|---|---|---|---|---|
| 3.902 | Upslope | 304.55 | 298.71 | 304.68 | 304.50 |
| 3.971 | Upslope | 304.30 | 299.76 | 304.54 | 304.46 |
| 4.040 | Apex | 304.37 | 302.53 | 304.42 | 304.32 |
| 4.087 | Downslope | 304.35 | 303.51 | 304.46 | 304.35 |
| 4.235 | Downslope | 304.51 | 303.75 | 304.57 | 304.56 |

**Table 2**
Assay of mefenamic acid in a solid dosage form (mg/unit dose)

| | Assay 1 | Assay 2 | Average | Theory | % Recovery |
|---|---|---|---|---|---|
| Formula 1 | 251.2 | 254.7 | 253.0 | 250 | 101.2 |
| Formula 2 | 253.2 | 249.8 | 251.5 | 250 | 100.6 |
| Formula 3 | 248.1 | 247.7 | 247.9 | 250 | 99.2 |
| Formula 4 | 245.5 | 249.1 | 247.3 | 250 | 98.9 |

claim. The average recovery was 100.7% (RSD = 0.8%).

*Assay of mefenamic acid in a new (proprietary) dosage form*

Four solid formulations were assayed in duplicate for mefenamic acid. The solid dosage forms were reduced to a powder using mortar and pestle. Samples containing approximately 250 mg of mefenamic acid were weighed and transferred to 100-ml volumetric flasks. To each flask 20 ml of 0.5 N NaOH solution was added and the mixture was agitated in the ultrasonic bath for 30 min. Solutions were made up to volume with water and 2-ml samples were diluted further to 100 ml with the preparation solvent. Samples were filtered through Millipore filters and 20-μl aliquots were injected into the chromatographic system. The results are presented in Table 2, which shows that the recovery of drug from the four formulae studied was 98.9–101.2%.

## Conclusions

Although spectrophotometric [19] and chromatographic [20–25] methods for determination of mefenamic acid in a variety of matrices have been reported, none of them was validated with respect to assay specificity regarding potential degradation products. In this method, the diode array detector with the numerical output of the purity parameter was employed to validate the specificity of the method. Use of this parameter showed a previous method to be non-specific and led to development of an improved specific method. This method is suitable for stability studies on dosage forms containing mefenamic acid.

## References

[1] S.M. Ficarro and K.A. Shah, *Pharm. Manuf.* **Sept.**, 25–27 (1984).
[2] *Pharmacopeial Forum* **9**, 2789–2792 (1983).
[3] D.R. Williams, *J. Assoc. Off. Analyt. Chem.* **69**, 432–436 (1986).
[4] E. Debesis, J.P. Boehlert, T.E. Givand and J.C. Sheridan, *Pharm. Tech.* **6**, 120–137 (1982).
[5] J. Guerra, *Pharm. Tech.* **March**, 74–78 (1988).
[6] M.J. Finkelson, *Pharm. Tech.* **March**, 75–84 (1988).
[7] Anon, *Pharmacopeial Forum* **12**, 1241–1245 (1986).
[8] R.J. Darnowski, *Pharmacopeial Forum* **11**, 941–943 (1985).
[9] H.C. Hamaker, *J. Assoc. Off. Analyt. Chem.* **69**, 417–418 (1986).
[10] J.A. Tetlow and D.T.E. Hunt, *J. Assoc. Off. Analyt. Chem.* **69**, 411–417 (1986).
[11] K. Florey, *S.T.P. Pharma.* **2**, 153–157 (1986).
[12] R. Dennis, *Pharm. Ind.* **Nov.**, 275–279 (1985).
[13] R.B. Taylor and A.S.H. Shivji, *Pharm. Res.* **4**, 177–180 (1987).
[14] R.B. Taylor, *Pharm. Res.* **5**, 250 (1988).
[15] L. Chafetz, *J. Pharm. Sci.* **60**, 335–345 (1971).
[16] L. Chafetz, *Pharm. Res.* **5**, 249 (1988).
[17] M.A. Tompkins, *Varian Instrument Division, Tech. Bull.*, 85–104.
[18] T. Alfredson and T. Sheehan, *J. Chromat. Sci.* **24**, 473–482 (1986).
[19] C.-F. Lim, K.N. Wynne, J.M. Barned, D.J. Topliss and J.R. Stockigt, *J. Pharm. Pharmac.* **38**, 795–800 (1986).
[20] C.K. Lin, C.S. Lee and J.H. Perrin, *J. Pharm. Sci.* **69**, 95–97 (1980).
[21] H.M. Stevens and R. Gill, *J. Chromat.* **370**, 39–47 (1986).
[22] H.J. Battista, G. Wehinger and R. Henn, *J. Chromat.* **345**, 77–89 (1985).
[23] L.J. Dusci and L.P. Hackett, *J. Chromat.* **172**, 516–519 (1979).
[24] C.I. Omile and I.R. Tebbett, *Chromatographia* **22**, 187–188 (1986).
[25] D.W. Hill and K.J. Langner, *J. Liq. Chromat.* **10**, 377–409 (1987).

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