Journal of Chromatography, 337 (1985) 201—202
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2359

Letter to the Editor

High-performance liquid chromatographic method for the simultaneous determination of myocardial creatine phosphate and adenosine nucleotides*

Sir,

In their recent note on the simultaneous analysis of myocardial creatine phosphate and adenine nucleotides, Bedford and Chiong [1] refer to our methodology "before publication". We would like to point out that our work, which is quite similar, has already been published, in 1982 in this journal [2], a fact ignored by the above authors. Our high-performance liquid chromatographic method did not work in their hands, which could be due to ultraviolet-absorbing impurities in the potassium dihydrogen phosphate, which vary from batch to batch. When we analyse small heart biopsies, we routinely purify this salt as described by Karkas et al. [3], using a Chelex-100 column (Bio-Rad Labs., Richmond, CA, U.S.A.). Alternatively, we substitute ammonium dihydrogen phosphate (Merck, Darmstadt, F.R.G.) for the potassium dihydrogen phosphate in buffer B, and use ammonium hydroxide (Merck) instead of potassium hydroxide for the preparation of buffer A [2]. For the analysis of freeze-clamped rat hearts, however, this purification or substitution was never necessary.

Bedford and Chiong [1] replaced half of the potassium dihydrogen phosphate by potassium chloride to avoid salt precipitation in the chromatographic system. We clean our chromatographs twice a month with water to avoid this problem (which is likely to occur also with potassium chloride). Besides, solutions containing halide salts should be avoided as mobile phases, since components made from stainless steel are subject to their attack [4].

The separation with the modified system [1] is hardly an improvement. Only half of the creatine phosphate peak disappears with the enzyme shift method (cf. Fig. 1B and D in ref. 1), indicating that this compound coelutes with substantial amounts of an unknown one.

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*For reasons of consistency in indexing and abstracting systems, the title conforms to the original publication by Bedford and Chiong [1]. The authors want to point out, however, that they consider "adenosine nucleotide" incorrect, and that they strongly prefer the term "adenine nucleotide".

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In conclusion, the modification proposed by Bedford and Chiong [1] for the analysis of myocardial creatine phosphate and adenine nucleotides does not seem to offer advantages compared to our original method [2].

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(Received August 3rd, 1984)