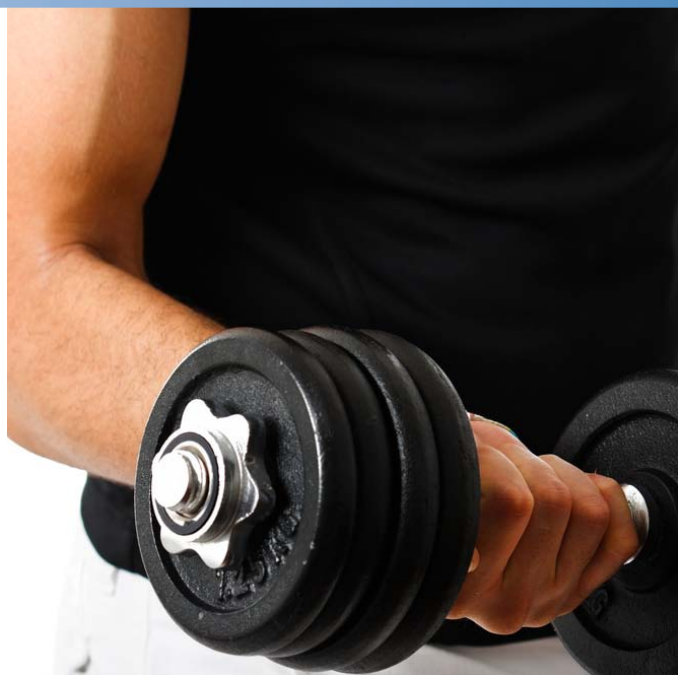
Analysis of Creatine and Creatinine on a Porous Graphitic Carbon Column by HPLC/UV

W. Faulkner, Thermo Fisher Scientific, Runcorn, Cheshire, UK

Key Words
Hypercarb, porous graphitic carbon, creatine, creatinine, polar compounds

Abstract
A simple and rapid reversed-phase HPLC/UV procedure for the examination of creatine and creatinine on a Thermo Scientific Hypercarb (porous graphitic carbon) column is described herein. Under reversed-phase, isocratic conditions, excellent separation of these polar analytes is achieved within five minutes and the chromatographic data exhibit excellent precision.



Introduction

Creatine [2-(methylguanidino) ethanoic acid] is a naturally occurring nitrogenous substance which is synthesized predominantly in the liver and kidneys and stored primarily in skeletal muscle as the high energy molecule phosphocreatine. Supplements of creatine are used sometimes by athletes and bodybuilders who wish to increase their muscular mass.

During its critical role in cellular metabolism, phosphocreatine is converted eventually to creatinine [2-amino-1-methyl-5H-imidazol-4-one] which is subsequently excreted in the urine. Thus, measurement of the rate of clearance of creatinine (via the glomerular filtration rate) can be used as an indicator of renal dysfunction.

Despite widespread utilisation of the non-specific Jaffe reaction [1,2] and enzymatic methods [3,4] for the determination of serum and urinary creatinine, chromatographic techniques (including ion-pair chromatography [5], ion-exchange chromatography [6] and, in particular, liquid chromatography-tandem mass spectrometry procedures [7,8]) continue to grow in popularity.

As a result of their high polarity, creatine and creatinine are unretained on traditional alkyl-bonded (e.g., C18) silicas. Moreover, the practical limitations inherent to both ion-pair and ion-exchange approaches have provided an impetus for the adoption of alternative chromatographic procedures (e.g., HILIC) and the development of new stationary phases (e.g., polar embedded and perfluorinated) that allow alternative mechanisms of interaction in addition to those dispersive interactions which are typically associated with alkyl-immobilised phases.

Hypercarb™ is a chromatographic support comprised of 100 % porous graphitic carbon. Hypercarb behaves as a strongly retentive alkyl-bonded silica for hydrophobic analytes, but its retentivity and selectivity towards polar and structurally-related compounds (e.g., diastereomers and geometric isomers) is substantially different [9].

The purpose of this investigation is to illustrate the effectiveness of Hypercarb for the retention and separation of the polar molecules creatine and creatinine under reversed-phase conditions.

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Experimental Details

Consumables	Part Number
Fisher Scientific water (HPLC gradient grade)	W/0106/17
Fisher Scientific acetonitrile (HPLC grade)	A/0626/17
Trifluoroacetic acid (TFA, HPLC grade, 99+ %)	T/3258/PB05
Fisher Scientific Finnpipette F2 pipettor kit (10 µL - 100 µL, 100 µL - 1000 µL, 1 ml - 10 mL)	PMP-020-220F
Fisher Scientific Finntip pipette tips, 10 µL	PMP-107-110W
Fisher Scientific Finntip pipette tips, 200 µL	PMP-107-600F
Fisher Scientific Finntip pipette tips, 1000 µL	PMP-103-206K
Fisher Scientific Finntip pipette tips, 10 mL	PMP-107-040R
Thermo Scientific borosilicate glass vials (2 mL, 12 mm x 32 mm) with 8 mm black screw cap fitted with a silicone/PTFE seal	60180-600
Creatine monohydrate (> 99 %) supplied by Fluka Analytical	
Creatinine (anhydrous, > 99 %) supplied by Sigma-Aldrich	

Sample Preparation	
Analytical standards	Primary analytical standards of both creatine and creatinine were prepared separately in water at a concentration of 1000 µg/mL. Thereafter, a mixed working standard was prepared in mobile phase (96.95:3:0.05 (v/v) H2O/MeCN/TFA) by combining 5 parts of creatine primary standard, 1 part creatinine primary standard and 94 parts of mobile phase. The concentrations of creatine and creatinine were 50 µg/mL and 10 µg/mL respectively.

Separation Conditions		Part Number
Instrumentation:	Thermo Scientific HPLC system equipped with a photodiode array (PDA) detector	
Column:	Thermo Scientific Hypercarb 5 µm, 100 mm x 2.1 mm	35005-102130
Mobile phase:	H_2O/MeCN/TFA (96.95:3:0.05 v/v)	
Flow rate:	0.2 mL/min	
Column temperature:	30 °C	
Autosampler temperature:	20 °C	
Detection:	UV at 216 nm	
Injection volume:	5 µL	
Syringe flush:	mobile phase	
Run time:	5 minutes	

Results

Under the reversed-phase, isocratic conditions adopted for this analysis the selectivity of the Hypercarb phase affords excellent resolution between these polar species in less than 5 minutes.

The repeatability in performance of the porous graphitic phase for the chromatographic examination of creatine and creatinine is summarised in Table 1. It is evident that the data are matched with excellent precision.

Analyte	k'		Efficiency (USP plates/m)		T_f (USP)		Resolution (USP)	
	mean	%RSD	mean	%RSD	mean	%RSD	mean	%RSD
Creatine	1.12	0.06	31602	0.90	1.33	1.07	n/a	n/a
Creatinine	2.44	0.05	41124	0.82	1.48	1.02	4.81	1.95

Table 1: Chromatographic performance of Hypercarb
k' - retention factor, T_f - tailing factor
Statistical assessment based upon data derived from 10 replicate injections

A typical chromatogram derived from the inspection of a solution of the polar analytes is shown in Figure 1.

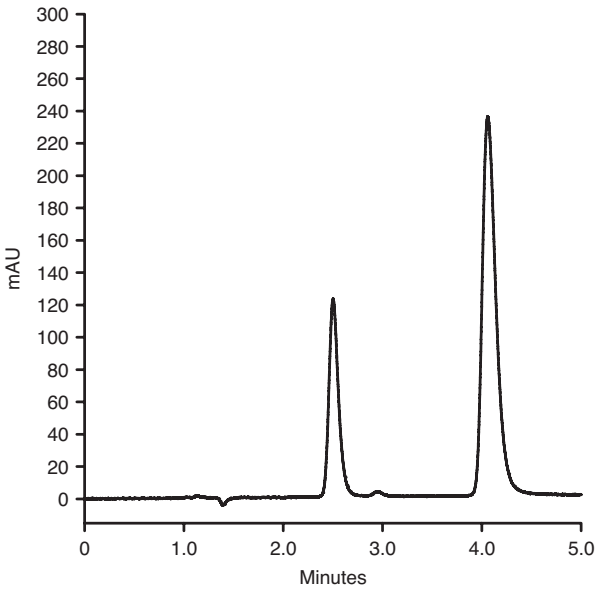

Figure 1: Separation of creatine (1) and creatinine (2) on Hypercarb

Conclusion

Hypercarb may be used for the successful chromatographic examination of creatine and creatinine. Under reversed-phase, isocratic conditions, excellent separation of these polar analytes can be achieved within five minutes.

In addition to normal dispersive interactions, the polar functional groups within both creatine and creatinine exhibit an affinity for the polarisable graphitic surface. This unique behaviour makes Hypercarb well suited to the retention and separation of both very polar and ionic species.

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+86 20 83145199 800 810 5118
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Technical Support
North America +1 800 332 3331
Outside North America +44 (0) 1928 534 440

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