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Letter to the Editor
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The Effect of Rasburicase on the Labile Fraction of Hemoglobin A1c During High Performance Liquid Chromatography

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Keywords

HPLC; Rasburicase; HbA1C

Letter to the Editor

We describe an unusual and previously unreported effect on High Performance Liquid Chromatography (HPLC) trace caused by rasburicase, the recombinant form of urate oxidase. A two-year-old boy presented with a mediastinal mass and was diagnosed to have a high white cell count T-cell acute lymphoblastic leukemia. He was noted to have a slightly reduced Mean Corpuscular Volume (MCV) of 73 fl (normal 75 to 87 fl) with normal ferritin levels. Unexplained microcytosis is investigated further with HPLC in our laboratory. The blood sample was therefore screened on a Bio-Rad Variant II HPLC analyzer® using the Bio-Rad Beta-thalassemia Short Program Assay® in order to exclude a possible thalassemia trait. An unexpected high peak was identified in the position associated with the labile fraction of glycated hemoglobin (HbA1c), with a retention time of 1.22 minutes (Figure 1A). The atypical peak was further investigated by HPLC of the original blood sample using the Bio-Rad Variant II HbA1c assay program (Figure 1B) which confirmed that the unusual peak eluted with the labile fraction of HbA1c. There were no obvious reasons to account for this and a likely association with the administration of rasburicase was considered. The boy had already had several sequential daily blood tests and thus an earlier sample taken prior to administration of rasburicase could be tested by HPLC using the Beta-thalassemia Short Program. This sample did not show the presence of a raised peak in this position (Figure 2A). A fresh sample from the same patient taken 24 hours after rasburicase was given also showed no evidence of a raised labile fraction of HbA1c (Figure 2B). His random blood glucose levels had remained normal throughout these days.

One or more doses of rasburicase are electively administered by clinicians prior to initiation of chemotherapy when the risk for tumor lysis is high. It is known to result in hemolysis and

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	4.4	1.22	58983
P2	---	5.1	1.33	68137
Unknown	---	0.8	1.47	11032
P3	---	7.3	1.71	98654
Ao	---	79.9	2.44	1075093
A2	3.0	---	3.64	33124

Total Area: 1345022

F Concentration = %
A2 Concentration = 3.0 %

Analysis comments:

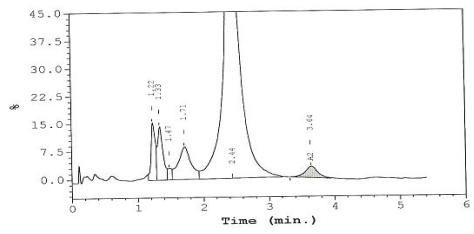

Figure 1A: Raised 'unknown' peak (4.4%; retention time 1.22 minutes) corresponding with labile fraction of HbA1C during rasburicase administration. The peak P2 (5.1%; retention time 1.33 minutes) is the stable fraction of HbA1C. P3 peak is also raised, indicating increased degradation changes.

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Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
A1a	---	3.7	0.20	68109
LA1c/CHb	---	5.5	0.70	101982
A1c	5.1	---	0.95	63504
P3	---	6.7	1.67	124917
Ao	---	80.6	1.80	1493282

Total Area: 1851794

A1c Concentration = 5.1 %

Analysis comments:

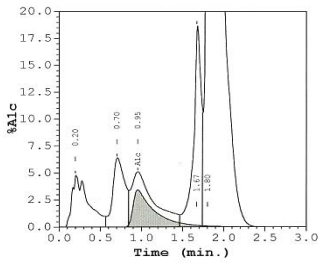

Figure 1B: Biochemical analysis confirming the rise in the labile fraction of HbA1C (5.5%; retention time 0.70 minutes).

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	1.1	1.26	17816
P2	---	4.8	1.33	79520
Unknown	---	0.9	1.47	15528
P3	---	5.7	1.70	94902
Ao	---	84.9	2.42	1412299
A2	3.1	---	3.63	42909

Total Area: 1662975

F Concentration = %
A2 Concentration = 3.1 %

Analysis comments:

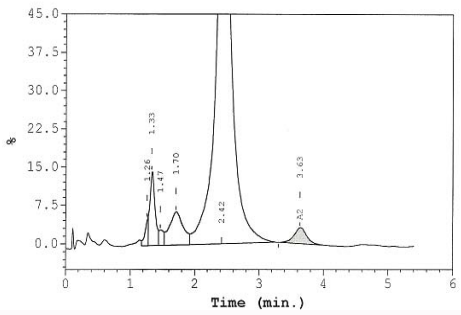

Figure 2A: Normal HPLC without the atypical peak pre rasburicase.

methemoglobinemia, especially in patients with concurrent G6PD deficiency [1,2]. Methemoglobin, unlike HbA1c, contributes to the region of P3 degradation product peak (Figure 1A), rather than a separate atypical peak. Rasburicase is also believed to impair carbohydrate metabolism and cause mild temporary elevation in blood glucose levels, usually not to a clinically significant degree [3]. This has manifested as an atypically raised HbA1c labile fraction

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	1.3	1.26	16254
P2	---	4.8	1.33	60983
Unknown	---	0.9	1.47	11902
P3	---	5.7	1.71	71845
Ao	---	84.6	2.43	1069717
A2	3.1	---	3.63	33422

Total Area: 1264122

F Concentration = %
A2 Concentration = 3.1 %

Analysis comments:

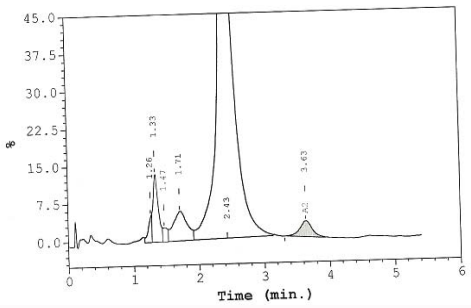

Figure 2B: Normal HPLC without the atypical peak 24 hours post rasburicase.

peak in this patient. This feature is time-dependent and transient. There should be no evidence of this atypical peak 24 hours after administration of rasburicase unless additional doses are given. Rasburicase is increasingly being used in hemato-oncology. Hematologists and biomedical scientists interpreting HPLC analysis should be aware of the above effects of rasburicase on the tracings due to subtle interference with carbohydrate metabolism, albeit the fact that this may not have clinical significance. It should also be noted that the peak due to the labile fraction of HbA1C depends on the age of the blood sample and is known to merge with the peak of the stable fraction if HPLC is performed after a few days. All our samples were tested less than 48 hours from phlebotomy and ideally should be analyzed as fresh as possible. It may be noted that we confirmed that the atypical peak was due to a genuine rise in HbA1c with the Bio-Rad HbA1c program, which uses a weak cation exchange column similar to the initial Bio-Rad Beta-thalassemia Short Program Assay*. Ideally in a prospective evaluation this confirmation should be done by an assay which uses a completely different methodology such as an affinity column method or mass spectrometry.

References

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