Acta Univ. Sapientiae, Alimentaria, **1** (2008) 49–60

Mercaptoethanesulfonic acid as the reductive thiol-containing reagent employed for the derivatization of amino acids with o-phthaldialdehyde analysis

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Abstract. Mercaptoethanesulfonic acid (MES-OH) can be applied for hydrolyzing proteins. The aim of our research was to examine if it can be used also as a derivatization reagent for fluorescens detection of amino acids together with OPA (*o*-phthaldialdehyde) instead of ME (mercaptoethanol) owning to its thiol-group. Corn, soybean and meatmeal samples were hydrolized with hydrochloric acid or MES-OH, derivatized with ninhydrin or OPA/ME or OPA/MES-OH and analysed with IEC (ion exchange chromatography) or RP-HPLC (reversed phase high performance liquid chromatography). There were no significant differences among the amino acid composition results of samples irrespective of choice of

Key words and phrases: Column liquid chromatography, reversed phase chromatography, hydrolysis of proteins, derivatization for fluorescence detection, mercaptoethanesulfonic acid

the hydrolysis or derivatization methods. MES-OH can be applied not only for hydrolysis but also for derivatization of amino acids. In case of samples with high protein content (>50%), due to the dilution after hydrolysis the MES-OH concentration could be insufficient for derivative formation, in these cases an extra MES-OH addition is required prior to derivatization.

1 Introduction

Hydrolysis of peptides and proteins with mercaptoethanesulfonic acid (MES-OH) has often been applied prior to determination of amino acid content of food and feed samples (with the exception of cysteine), and tryptophan content of samples with low amount of carbohydrates [4]. Side-chains of amino acids which are susceptible to oxidation during hydrolysis can be protected with the use of MES-OH due to its thiol group with reducing properties [2]. With the use of MES-OH oxidized forms of methionine were converted to methionine and the indol group of tryptophan was protected against oxidation [3]. Prior to HPLC analyses fluorescent derivatives can be formed by the reaction of amino acids with *o*-phthaldialdehyde (OPA) [1, 5] in the presence of a reductive thiol-containing reagent, such as 2-mercaptoethanol (ME) in order to improve the limit of detection. The aim of our research was to examine the possibility of using MES-OH not only for hydrolysis of proteins but also for derivatization of amino acids in the subsequent step before analysis by HPLC.

2 Experimental data

Hydrolysis of proteins. Two protein hydrolysis methods were applied:

1. Hydrolysis with hydrochloric acid: 5 ml 6 M hydrochloric acid addition to 100 mg sample and heating in a closed glass vessel at 110±1 °C for 24 h in nitrogen atmosphere.
2. Hydrolysis with MES-OH: 5 ml of 3 M MES-OH solution was added to 100 mg sample and heated in a closed inert vessel at 125±1 °C for 24 h in nitrogen atmosphere.

In case of hydrolysis with hydrochloric acid, the required pH values of the solutions for IEC analysis (pH = 2.2) and for derivatization prior to HPLC analysis (pH = 7.0) were set with 4 M sodium hydroxide solution. After hydrolysis samples were diluted, filtered and frozen at −24 °C. Before analyses samples were filtered through 0.45 μm hidrofil membrane-filters.

Derivatization and analysis. IEC analyses were carried out with an amino acid analyzer (Aminochrom OE-914, LaborMIM, Hungary). Amino acids were separated on a cation-exchange column (230 mm × 4.5 mm, "Kemochrom 9" resin), postcolumn ninhydrin derivatives of amino acids were detected with an on-line fotometer at 570 nm.

Prior to HPLC analyses samples were precolumn derivatized with OPA/ME or OPA/MES-OH with the programmable autosampler. First 200 μl (0.4 M; pH = 9.5) borate buffer was added to 450 μl sample solution, then 100 μl derivatization reagent (100 mg OPA and 100 μl ME /or 100 μl 3 M MES-OH/ in 9 ml methanol and 1 ml borat buffer) was added and mixed. After three min 20 μl of the mixture was injected. The HPLC analyses were accomplished with a MERCK-Hitachi HPLC comprising L-7250 programmable autosampler, L-7100 pump, L-7350 column thermostat, L-7480 fluorescence detector, and AIA data conversion utility for the D-7000 HPLC system manager. The compounds were separated on a 250 mm × 4 mm column packed with Superspher 60 RP-Select B (MERCK, Darmstadt, Germany), the temperature of thermostat was 40 °C. The composition of the mobile phase are shown on *Table 1.* The derivatives were detected with a fluorescence detector (λ_{ex} 330 nm, λ_{em} 450 nm). Reagents were p. a. grade. Solvents (methanol and acetonitril) were HPLC gradient grade and purchased from MERCK (Darmstadt, Germany).

Table 1: Eluent composition applied for the separation of OPA-MES-OH-amino acid derivatives[1]

Time (min)	**Metanol (v/v%)**	**Na-acetate solution**[2] **(v/v%)**	**Acetonitril (v/v%)**
0	4	96	0
10	10	90	0
27	11	84	5
28	8	87	5
37	12	81	7
59	16	68	16
60	18	37	45
71	4	96	0
75	4	96	0

[1]The flow rate was 1 ml/min
[2]50 mM (pH 7.0)

3 Results

Chromatograms of the OPA/MES-OH derivatives of amino acids in standards and in corn, soybean and meatmeal samples can be seen in *Figs 1-4.*

Figure 1: Separation of OPA/MES-OH derivatives of amino acids of a standard solution carried out by RP-HPLC with the gradient shape can be seen at *Table 1*

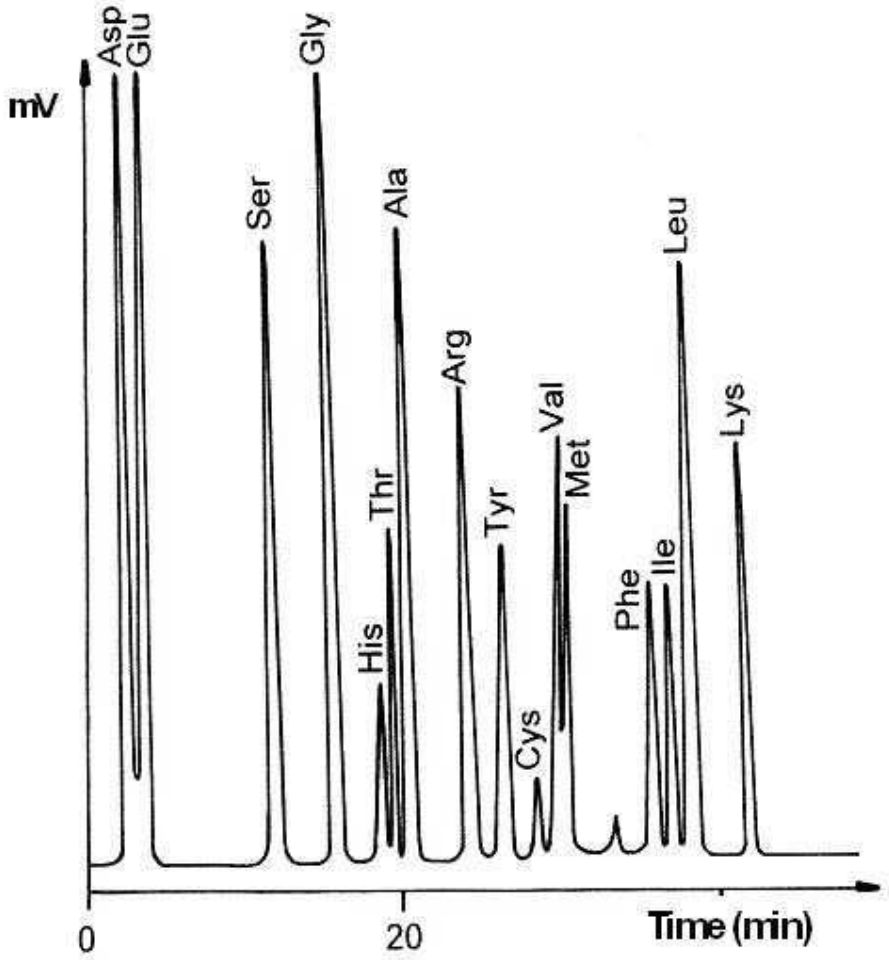


Figure 2: Separation of OPA/MES-OH derivatives of amino acids of a corn sample (see details in *Fig 1)*

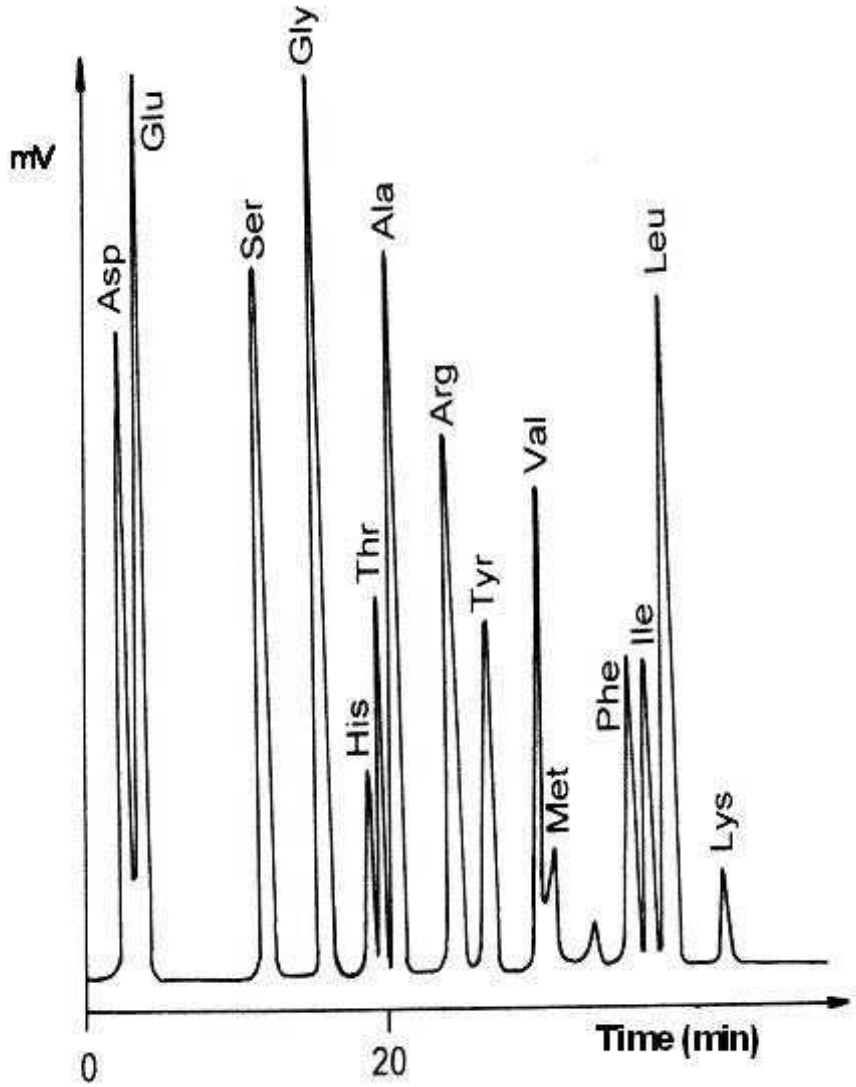


Figure 3: Separation of OPA/MES-OH derivatives of amino acids of a meatmeal sample (see details in *Fig 1)*

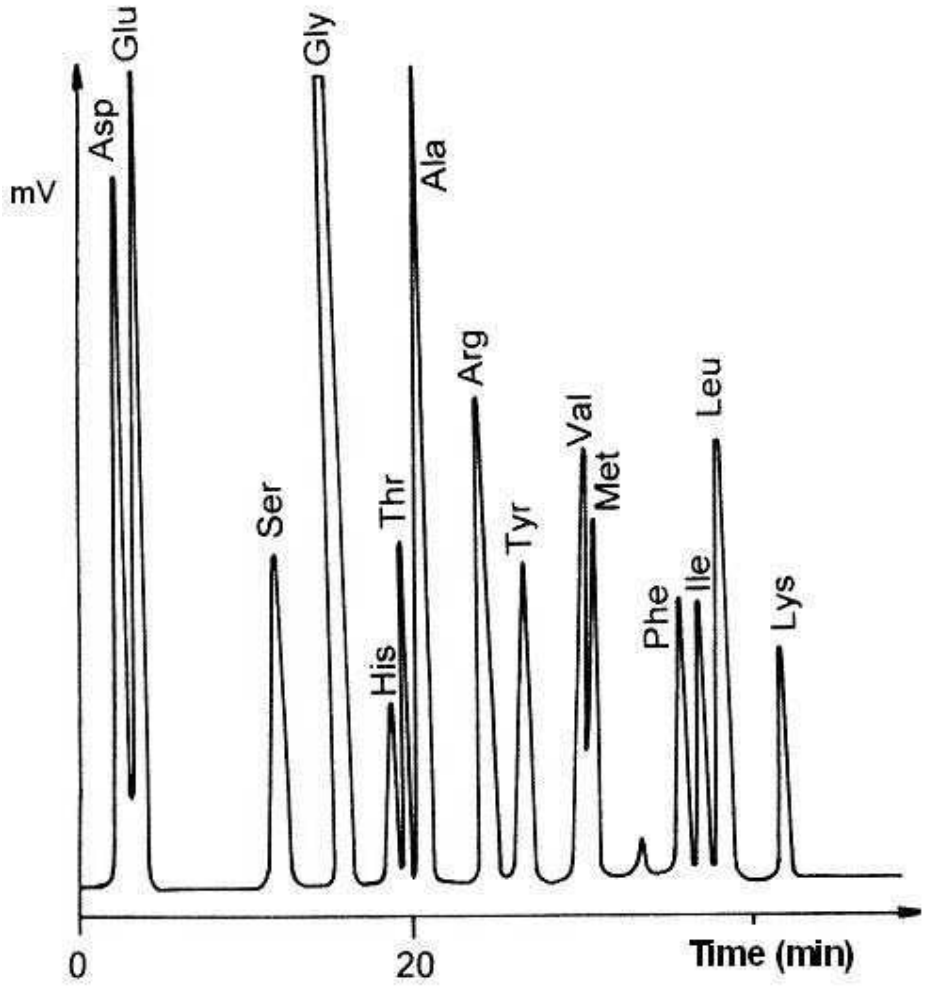
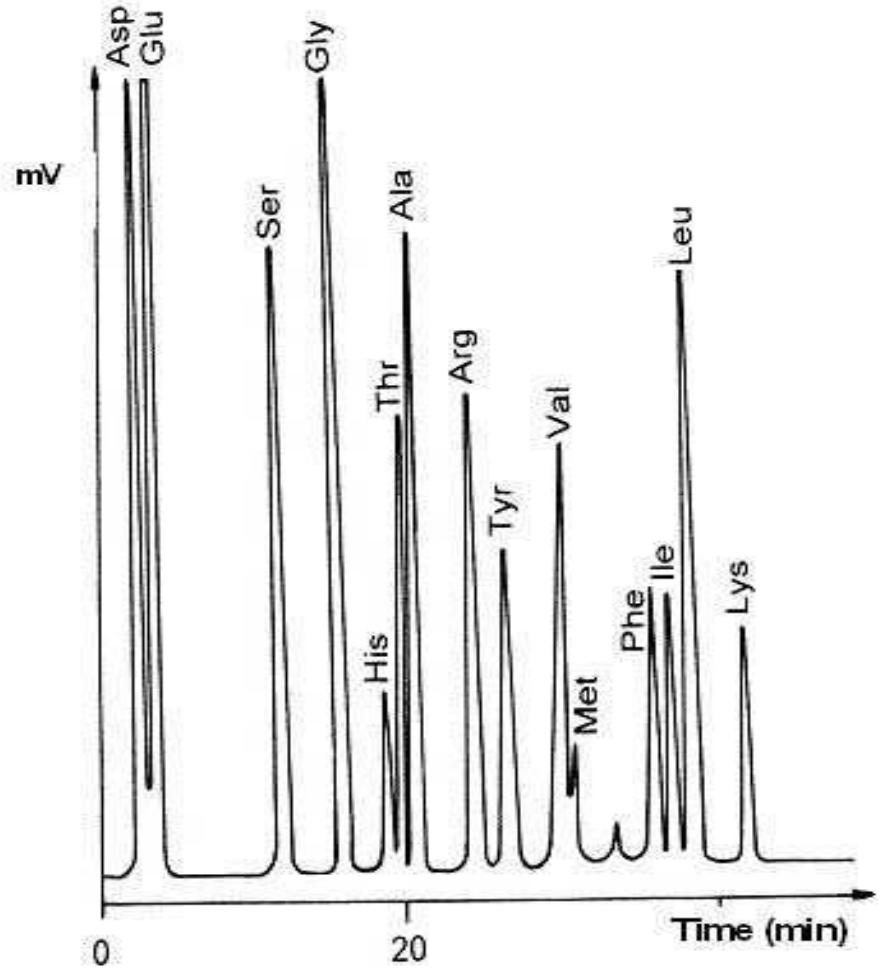


Figure 4: Separation of OPA/MES-OH derivatives of amino acids of a soybean sample (see details in *Fig 1)*



When the OPA/MES-OH derivatization procedure was applied, the sensitivity was about five-fold higher than in case of OPA/ME-method. The amino acid composition of corn, soybean and meatmeal samples analyzed by IEC and HPLC, and hydrolized and derivatized with different methods are shown in *Table 2–4*, respectively.

Table 2: Amino acid content of corn (g/100 g sample) determined with IEC (postcolumn ninhydrin derivatization) and with HPLC (derivatization with OPA/ME after hydrochloric acid hydrolysis; derivatization with OPA/MES-OH without[1] or with[2] an extra MES-OH addition after a MES-OH hydrolysis)

Instrumentation	**IEC**	**HPLC**		
Hydrolysis	**HCl**		**MES-OH**	
Derivatization	**ninhydrin**	**OPA/ME**	**OPA[1]**	**OPA/ MES-OH[2]**
Asp	0.66	0.70	0.69	0.71
Thr	0.32	0.34	0.36	0.36
Ser	0.42	0.47	0.49	0.48
Glu	1.84	1.84	1.82	1.83
Gly	0.34	0.38	0.36	0.37
Ala	0.57	0.61	0.60	0.59
Val	0.42	0.50	0.47	0.49
Met	0.19	0.21	0.23	0.24
Ile	0.36	0.37	0.39	0.40
Leu	0.93	0.94	0.92	0.94
Tyr	0.32	0.36	0.37	0.36
Phe	0.38	0.36	0.37	0.38
Lys	0.29	0.28	0.30	0.29
His	0.22	0.24	0.23	0.25
Arg	0.48	0.54	0.52	0.51

Table 3: Amino acid content of soybean (g/100 g sample) determined with IEC (postcolumn ninhydrin derivatization) and with HPLC (derivatization with OPA/ME after hydrochloric acid hydrolysis; derivatization with OPA/MES-OH without[1] or with[2] an extra MES-OH addition after a MES-OH hydrolysis)

Instrumentation	**IEC**	**HPLC**		
Hydrolysis	**HCl**		**MES-OH**	
Derivatization	**ninhydrin**	**OPA/ME**	**OPA[1]**	**OPA/ MES-OH[2]**
Asp	5.68	5.72	5.69	5.70
Thr	1.63	1.53	1.72	1.74
Ser	2.12	2.35	2.36	2.41
Glu	9.29	9.14	9.23	9.19
Gly	2.14	1.97	2.06	2.11
Ala	1.90	1.98	1.94	1.96
Val	1.90	2.20	1.99	1.98
Met	0.61	0.59	0.68	0.72
Ile	1.73	1.94	1.84	1.86
Leu	3.32	3.17	3.29	3.26
Tyr	1.78	1.55	1.81	1.83
Phe	2.12	2.05	2.07	2.11
Lys	2.96	2.82	2.93	2.92
His	1.19	1.15	1.17	1.20
Arg	3.03	3.09	3.02	3.07

Table 4: Amino acid content of meatmeal (g/100 g sample) determined with IEC (postcolumn ninhydrin derivatization) and with HPLC (derivatization with OPA/ME after hydrochloric acid hydrolysis; derivatization with OPA/MES-OH without[1] or with[2] an extra MES-OH addition after a MES-OH hydrolysis)

Instrumentation	IEC	HPLC		
Hydrolysis	HCl		MES-OH	
Derivatization	ninhydrin	OPA/ME	OPA[1]	OPA/ MES-OH[2]
Asp	3.93	3.85	3.92	3.90
Thr	1.42	1.39	1.43	1.44
Ser	1.60	1.62	1.60	1.59
Glu	5.94	6.02	5.97	6.01
Gly	6.92	6.72	6.83	6.79
Ala	3.98	3.95	3.91	3.97
Val	1.89	2.26	2.16	2.21
Met	0.75	0.73	0.84	0.82
Ile	1.29	1.33	1.31	1.31
Leu	2.65	2.64	2.67	2.63
Tyr	1.06	1.04	1.22	1.21
Phe	1.44	1.36	1.41	1.42
Lys	2.40	2.33	2.37	2.41
His	0.79	0.81	0.82	0.81
Arg	3.32	3.31	3.34	3.29

In fact, there were no significant differences among the results of IEC and HPLC analyses irrespective of choice of the hydrolysis or derivatization methods. Although in case of samples analyzed by HPLC and derivatized with the OPA/ME or with the OPA/MES-OH methods the concentration of some amino acids (threonine, serine, methionine, tyrosine) were slightly higher than in case of IEC analysis with ninhydrin derivatization. When samples were hydrolized with MES-OH, in most cases the concentration of MES-OH after dilution was enough also for derivatization and therefore the addition of OPA was solely required. If the solution after hydrolysis contains at least 1 μl of 3 M MES-OH that is, the protein content of the sample was below 50%, an extra addition of MES-OH was not necessary for derivatization. In case of

corn samples with crude protein content of 9-10% the amount of MES-OH in diluted sample (approx. 4 μl 3 M) was sufficient for derivatization, and also for meatmeal and soybean samples with 46.6–45.2% crude protein content. If the protein content of the sample is less than 5% the peak of MES-OH can disturb the evaluation of acidic amino acids in IEC.

The intensity of OPA/MES-OH derivatives on chromatograms began to decrease after 10–12 hours of the reaction, therefore analysis should be achieved within this time period. After that time a significant decrease was detected mostly in case of aspartic acid and glutamic acid derivatives.

4 Acknowledgements

This work has been accomplished with the financial support of OTKA (National Foundation for the Subsidy of Research, Hungary). The number of the contract is T061587. The Sapientia–Hungarian University of Transylvania, Institute of Research Programs, also granted the research. Their financial assistance is gratefully acknowledged.

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Received: August, 2008