CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

082004

The AOAC Research Institute hereby certifies the method known as:

SafTest Percent Fat

manufactured by

MP Biomedicals
29525 Fountain Parkway
Solon, Ohio 44139
USA

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*[SM] Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[SM] certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

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METHOD NAME
SafTest Percent Fat

CATALOG NUMBER
07KTPF2000

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APPLICABILITY OF METHOD

Analyte – Fat content as % fat in grams (g) determined as triglyceride per 100 grams of test matrix.

Matrixes – (1 g) – Potato chips and grain-based snack products; and meat meal and fishmeal products.

Performance claims – The practical dynamic range of the determination was established as 0.04 to 0.33 grams(g) fat determined as triglycerides in 100 grams of these matrixes. Higher fat content levels up to 50% fat can be tested with an appropriate dilution. The limit of quantitation was determined as 0.06% fat for low-fat lipid solutions run neat (0.06 % fat is the low calibrant) and 0.24 % for most materials diluted 1:4 during solubilization. (Fish meal and crackers were 0.28% and 0.36%, respectively). Mean recoveries from replicate analyses of triglyceride solutions at three concentrations were 101 to 103 % with relative standard deviation of repeatability (RSD_r) below 5%. Spike recoveries of triglycerides from liquid egg whites, cracker, pretzels and chicken meals ranged from 99.8% to 108%. Studies are underway to determine if an acid hydrolysis step prior to testing is necessary for complete recovery of fat from fish meal. Glycerol produces interference with this method. Glycerol content below 5 % can be corrected by subtraction.

REFERENCE METHODS

Official Methods and Recommended Practices of the AOCS (1996) 11th ed. AOCS, Method Aa 4-38 (2)

Official Methods and Recommended Practices of the AOCS (1996) 11th ed. AOCS, Method Am 5-04. (3)

Codex Alimentarius Commission. *Procedural Manual*, 24th ed. Published online at www.codexalimentarius.org/procedures- strategies/procedural-manual/en/. FAO, Rome, 2015. (4)

ORIGINAL CERTIFICATION DATE
August 24, 2020

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2024.

METHOD MODIFICATION RECORD
NONE

SUMMARY OF MODIFICATION
NONE

Under this AOAC *Performance Tested Methods*SM License Number, 082004 this method is distributed by:
NONE

Under this AOAC *Performance Tested Methods*SM License Number, 082004 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)

The SafTest System is a series of micro-analytical techniques based on membrane separation technology as an alternative to sensory and traditional analytical methods. One test of the series the SafTest Percent Fat test measures fat content. Fat content is used to convert free fatty acid and peroxide values to a fat basis as well as monitoring raw material ingredient contents.

The Percent Fat test is designed to measure the fat content in prepared test portions using enzymatic hydrolysis of triglycerides to glycerol, followed by an enzymatic and colorimetric determination of the glycerol released. For liquid materials, test portions are solubilized in the proprietary preparation reagent, reacted with reagent and read with an optical reader. For dry materials, test portions are solubilized, separated through the membrane separation pack, reacted with reagent and read with an optical reader.

This test procedure is intended to accurately measure percent fat levels above the LOQ of 0.06% for liquid materials run neat and 0.24 % for dried food products diluted 1:4. However, food products with higher levels such as nut butters can be easily tested with a dilution step prior to analysis. The SafTest Percent Fat Test produces reliable results with large dilutions up to 1:100.

DISCUSSION OF THE VALIDATION STUDY (1)

The practical dynamic range of the determination was established as 0.04 to 0.33 grams(g) triglyceride in 100 grams of these matrixes. Higher fat content levels can be evaluated with a dilution. The LOQ was determined as 0.06% for low fat lipid solutions run neat and 0.24 % for most materials diluted 1:4 during solubilization. The LOQs for fish meal and crackers were slightly higher at 0.28% and 0.32%, respectively

Mean recoveries from replicate analyses of triglyceride solutions at three concentrations yielded 101 to 103 % with RSD_rs below 5%. The average recoveries from all matrix studies ranged from 88.8% to 116.2% when calculated as a % of reference method results. Reference methods were often approved because the availability of equipment in the contract and independent laboratories and their familiarity with the methods. The choice of reference method would have a large impact on recovery.

Overall matrix studies, the repeatability of the SafTest Percent Fat Test was found to be in the range 1.4 to 7.2 %, which is well within performance expectations for this assay and overall an improvement over the reference methods which ranged from 1.1% to 21.5%.

Fatty amides, amines, alcohols, aldehydes, esters, anhydrides, and acetates with the exception of glycerol produced no interferences. Glycerol was found to be almost completely recovered and interferes with the SafTest Percent Fat test method. Solutions of varying amounts and mixtures of monoglycerides, diglycerides and triglycerides gave recoveries ranging from 99.9% to 102% relative to AOCS Aa 4-38. Recovery of a phospholipid was 0% which is also similar to AOCS Aa 4-38, which also had no recovery of phospholipid.

Limitation of the method include:

- Inorganic acids or bases in food matrixes can interfere with the SafTest Percent Fat Test by inactivating the enzymes in Reagent A. Materials being tested should be in the pH range of 5 to 8.
- There are some applications when concentrations below the LOQ of 0.24% in solubilized food materials would be of interest.
- Recoveries were inconsistent for meat meals and appear to be product dependent. It is critical that the preparation of meals with saturated fat is performed at the higher temperature (46-50ºC), and if the fat composition is unknown the higher temperature should be used.
- Low recoveries were observed for fish meals compared to the reference methods.

The differences seen in the results on potato chips in the independent laboratory study compared to the earlier training study results demonstrates the importance of handling high fat matrixes as recommended. The fishmeal results using SafTest Percent Fat test were lower than AOAC 948.16 that was specifically adapted for fishmeal. It is evident that a preparation step should be included in the Percent Fat Test to digest the fishmeal adequately for membrane separation as has been done in AOAC 948.16 (3) and this update is in progress.

Table 11. Method Developer Matrix Study on Potato Chips, Crackers and Other Grain-Based Snacks Study 1 (1)

Matrix	Method	N	Fat ,% [a]	s_r	RSD_r %	Bias[b]	Recovery[c], %
Potato chip	AOCS Aa 4-38	3	33.8	0.53	1.6	-1.22	94.2
	SafTest Percent Fat	8	32.85	1.58	4.9		
Kettle potato chip	AOCS Aa 4-38	3	21.9	1.3	5.9	1.60	107.2
	SafTest Percent Fat	8	23.50	0.98	3.4		
Graham cracker	AOCS Aa 4-38	3	14.4	0.10	6.9	1.45	110.5
	SafTest Percent Fat	8	15.85	0.56	3.5		
Wheat cracker	AOCS Aa 4-38	3	12.33	0.24	1.1	0.56	104.9
	SafTest Percent Fat	8	12.89	0.61	4.7		
Round cracker	AOCS Aa 4-38	3	27.17	0.32	1.2	3.74	109.9
	SafTest Percent Fat	8	30.91	0.97	3.1		
Tortilla chip	AOCS Aa 4-38	3	23.05	0.55	2.3	-1.82	95.4
	SafTest Percent Fat	8	21.17	1.45	6.8		
Baked potato chip	AOCS Aa 4-38	3	9.70	0.29	3.0	1.46	114.9
	SafTest Percent Fat	8	11.16	0.81	7.2		
Sweet cracker	AOCS Aa 4-38	3	10.93	0.12	1.1	2.47	113.7
	SafTest Percent Fat	8	12.40	0.36	2.9		

[a] Mean Fat, %
[b] Bias calculated as Saftest Percent Fat Test result minus AOCS Aa 4-38 result
[c] Recovery of the Saftest Percent Fat Test expressed as a % of the AOCS Aa 4-38 result

Table 12. Method Developer Matrix Study on Potato Chips, Crackers and Other Grain-Based Snacks Study 2 (1)

Matrix/Lab ID	Method	N	Fat ,% [a]	s_r	RSD, %	Bias[b]	Recovery[c], %
Baked chips/#111S-118S	AOCS Aa 4-38	8	9.51	0.16	1.7	0.59	131.2
	SafTest Percent Fat	7	13.08	0.40	3.1		
Pretzels/ #121S –128S	AOCS Aa 4-38	8	2.34	0.15	6.4	0.1	95.6
	SafTest Percent Fat	8	2.24	0.09	4.0		
Crackers/#131S-138S	AOCS Aa 4-38	8	7.35	0.21	2.9	1.31	118.1
	SafTest Percent Fat	8	8.66	0.33	3.8		
Corn tortillas/#141S-148S	AOCS Aa 4-38	8	1.81	0.26	14.4[d]	0.35	120.0[d]
	SafTest Percent Fat	8	2.16	0.12	5.6		
Flour tortillas/#151S-158S	AOCS Aa 4-38	8	10.31	2.22	21.5[d]	4.21	NC[e]
	SafTest Percent Fat	8	14.52	0.50	3.4		
Corn strips/#161S-168S	AOCS Aa 4-38	8	16.89	0.61	3.6	3.52	120.0
	SafTest Percent Fat	8	20.41	0.47	2.3		

[a] Mean Fat, %
[b] Bias calculated as SafTest Percent Fat Test result minus AOCS Aa 4-38 result
[c] Recovery of the SafTest Percent Fat Test expressed as a % of the AOCS Aa 4-38 result
[d] Reference Method RSD> 20% so not included in recovery
[e] Not calculated

Table 13. Method Developer Matrix Study on Meat Meals, including Fish Meal (1)

Matrix/Lab ID	Method	N	Fat ,% [a]	s_r	RSD, %	Bias[b]	Recovery[c], %
Poultry meal/#111-118M	AOCS Aa 4-38	8	16.30	2.84	17.4	-1.20	92.4
	SafTest Percent Fat	8	15.10	0.35	2.3		
Chicken meal/ #121M-128M	AOCS Aa 4-38	8	15.81	3.42	21.6	-3.79	79.2
	SafTest Percent Fat	8	12.02	0.25	2.0		
Pork meal/#131M-138M	AOCS Aa 4-38	8	15.32	1.60	14.4	-1.85	88.5
	SafTest Percent Fat	8	13.47	0.19	1.4		
Chicken meal/ #141M-148M	AOCS Aa 4-38	8	13.86	0.61	4.5	-.90	93.5
	SafTest Percent Fat	8	12.96	0.35	2.4		
Animal meal/ #151M-158M	AOCS Aa 4-38	8	13.86	0.61	4.3	-.86	93.5
	SafTest Percent Fat	8	12.92	0.44	3.4		
Fish meal/#161M-168M	AOCS Aa 4-38	8	9.07	0.66	6.2	-2.63	71.2
	SafTest Percent Fat	8	6.47	0.24	2.8		
Lamb meal/#171M-178M	AOCS Aa 4-38	8	13.55	0.34	2.5	0.99	105.3
	SafTest Percent Fat	8	14.44	0.39	2.7		

[a] Mean Fat, %
[b] Bias calculated as Saftest Percent Fat Test result minus AOCS Aa 4-38 result
[c] Recovery of the Saftest Percent Fat Test expressed as a %

Table 15. Independent Laboratory Matrix Study (1)

			Fat, %[a]				
Matrix	n	Parameter	SafTest Percent Fat	Reference Method	Reference Method [b]	Recovery, %[c]	p value by t test
Crackers	8	Mean	26.64	AOCS Am 5-04	27.45	97	0.1275
		$\%RSD_r$	4.9		1.2		
Baked Corn Scoops	8	Mean	7.48 [b]	AOCS Am 5-04	8.01	93.4	0.0006
		$\%RSD_r$	2.7		3.3		
Potato Chips, Fried	8	Mean	33.29[b]	AOCS Am 5-04	36.87	90.3	0.0004
		$\%RSD_r$	2.6		4.7		
Chicken Meal	8	Mean	13.95	AOCS Am 5-04	13.85	100.8	0.6997
		$\%RSD_r$	3.9		4.1		
Meat & Bone Meal	8	Mean	8.13 [b]	AOCS Am 5-04	9.85	82.3	4.253×10^{-7}
		$\%RSD_r$	4.6		1.9		
Fish Meal	8	Mean	8.39[b]	AOAC 948.16	11.22	74.8	5.378×10^{-10}
		$\%RSD_r$	4.2		3.6		

[a]All values are an average of 8 independent replicates.
[b]Result statistically different from corresponding reference results ($p < 0.05$)

REFERENCES CITED

1. Gordon, V.C., Rainey, C.C., and Studmire, W.C., Validation of the SafTest Percent Fat for the Measurement of the Fat Content of Meat Meals, Fish Meal and Potato Chips, Crackers and Other Grain-Based Snack Products, AOAC *Performance Tested Methods*℠ certification number 082004.
2. Official Methods and Recommended Practices of the AOCS (1996) 11th ed. AOCS, Method Aa 4-38
3. Official Methods and Recommended Practices of the AOCS (1996) 11th ed. AOCS, Method Am 5-04.
4. Codex Alimentarius Commission. *Procedural Manual*, 24th ed. Published online at www.codexalimentarius.org/procedures- strategies/procedural-manual/en/. FAO, Rome, 2015.