

# Azo Dye Metabolite Standards



## Introduction

Azo-dyes are a large class of synthetic organic dyes that contain nitrogen in the form of an azo group ( $-N=N-$ ), as part of their molecular structures. They are used in many areas such as the food, cosmetic, textile, leather, nutrition, plastic and pharmaceutical industries. During the past 50 years, the amount of azo-dyes used in foods has increased by 500%. When compared to natural dyes, synthetic food dyes provide many advantages. Synthetic dyes are cheaper, more easily available, last longer and can achieve colour and hue variations otherwise not possible using natural colourants. They also provide superior colour fastness and colour intensity.

However, since the use of synthetic food colouring has become widespread, many allergic and other immune reaction disorders, have increasingly been reported. The reductive cleavage of the azo bond leads to the formation of aromatic amines which may be mutagenic, carcinogenic or allergenic. For instance, acid red 85 and direct blue 6, are both capable of reductively splitting to produce carcinogenic benzidine. Likewise, Sudan II and disperse yellow 7 are capable of splitting to form p-phenylenediamine and aniline, while disperse orange 3 can split only to p-phenylenediamine. <sup>(1)</sup>

## Legislation

Colour Directive 94/36/EC outlines the permitted natural and synthetic colours with their approved applications and limits in different foodstuffs (Commission, 1994) and the use of azo-dyes which can be reduced into toxic amines is prohibited in Europe, US and many other countries. The safety of food colours and other food additives in the EU is evaluated by the European Food Safety Authority (EFSA). Since 2009, the expert Scientific Panel of EFSA assess all of the permitted food colours (45 in total) which had been approved for use in the EU giving priority to those synthetically produced and then to those obtained from natural sources mainly carotenoids. Since new scientific data became available, there have been changes in the legislation, many additives which were initially authorised for used in the past, are currently not permitted in food products in the EU. Unfortunately, there are reports of food adulteration by using dyes unauthorised for food which are often hazardous.

## Illegal Adulteration

There have been many notifications from several EU Member States via the Rapid Alert System for Food and Feed (RASFF) of the occurrence of Sudan I, II, III and IV, para red, rhodamine b, and orange 2 in chilli and curry powder and processed products containing chilli or curry powder, sumac, curcuma and palm oil among others. There have also been occurrences of azo dyes released from clothing and textiles, which may be accidentally ingested intradermally or orally by people wearing such clothes. Textile workers are also at risk.

## Metabolite Standards

Efficient analytical methods for the determination of food colorants are of utmost importance since their illegal presence in food threatens consumer's safety. Up to now, most methods are focused to detect dyes so far found illegally present in food. There are no methods focused in the detection of aromatic amines derived from azo dyes which may potentially appear illegally in food and show carcinogenic effects in humans.

In a study funded by and participated in by scientists in Reagecon, we have taken account of this consideration and have tried to fill this void. For example, we have provided and published a rapid, accurate and precise method for the identification and quantification of various synthetic food colourant products in paprika. As always, our principle role has been to characterise, purify, validate and offer high quality standards for these products and disseminate these into the marketplace. Further details can be found at [www.reagecon.com](http://www.reagecon.com)

<sup>(1)</sup> Report 6/14 Chemicals in textiles - risks to human health and the environment. KEM Swedish Chemicals Agency, Stockholm 2014

Product No.	Analyte	Concentration & Matrix	Pack Size
REAZO001	2,4-Diaminoanisole	1000µg/ml in HPLC Water	1ml
REAZO002	2,4-Diaminoanisole	2000µg/ml in HPLC Water	1ml
REAZO003	2,4-Diaminotoluene	1000µg/ml in Purge & Trap Methanol	1ml
REAZO004	2,4-Diaminotoluene	2000µg/ml in Purge & Trap Methanol	1ml
REAZO005	3,3-Dichlorobenzidine	1000µg/ml in Purge & Trap Methanol	1ml
REAZO006	3,3-Dichlorobenzidine	2000µg/ml in Purge & Trap Methanol	1ml
REAZO007	3,3-Dimethoxybenzidine	1000µg/ml in Purge & Trap Methanol	1ml
REAZO008	3,3-Dimethoxybenzidine	2000µg/ml in Purge & Trap Methanol	1ml
REAZO009	3-Aminobiphenyl	1000µg/ml in Ethyl Acetate	1ml
REAZO010	3-Aminobiphenyl	2000µg/ml in Ethyl Acetate	1ml
REAZO011	4,4,-Diaminodiphenylmethane	1000µg/ml in Purge & Trap Methanol	1ml
REAZO012	4,4,-Diaminodiphenylmethane	2000µg/ml in Purge & Trap Methanol	1ml
REAZO013	4,4-Methylenebis(2-chloroaniline)	1000µg/ml in Purge & Trap Methanol	1ml
REAZO014	4,4-Methylenebis(2-chloroaniline)	2000µg/ml in Purge & Trap Methanol	1ml
REAZO015	4-Aminoazotoluene	1000µg/ml in Purge & Trap Methanol	1ml
REAZO016	4-Aminoazotoluene	2000µg/ml in Purge & Trap Methanol	1ml
REAZO017	4-Aminobiphenyl	1000µg/ml in Purge & Trap Methanol	1ml
REAZO018	4-Aminobiphenyl	2000µg/ml in Purge & Trap Methanol	1ml
REAZO019	4-Chloroaniline	1000µg/ml in Purge & Trap Methanol	1ml
REAZO020	4-Chloroaniline	2000µg/ml in Purge & Trap Methanol	1ml
REAZO021	5-Nitro-o-toluidine	1000µg/ml in Purge & Trap Methanol	1ml
REAZO022	5-Nitro-o-toluidine	2000µg/ml in Purge & Trap Methanol	1ml
REAZO023	Anilazine	1000µg/ml in Purge & Trap Methanol	1ml
REAZO024	Anilazine	2000µg/ml in Purge & Trap Methanol	1ml
REAZO025	Azobenzene	1000µg/ml in Purge & Trap Methanol	1ml
REAZO026	Azobenzene	2000µg/ml in Purge & Trap Methanol	1ml
REAZO027	Benzidine	1000µg/ml in Purge & Trap Methanol	1ml
REAZO028	Benzidine	2000µg/ml in Purge & Trap Methanol	1ml
REAZO029	Dimethylaminoazobenzene	1000µg/ml in Purge & Trap Methanol	1ml
REAZO030	Dimethylaminoazobenzene	2000µg/ml in Purge & Trap Methanol	1ml
REAZO031	o-anisidine	1000µg/ml in Purge & Trap Methanol	1ml
REAZO032	o-anisidine	2000µg/ml in Purge & Trap Methanol	1ml
REAZO033	o-Toluidine	1000µg/ml in Purge & Trap Methanol	1ml
REAZO034	o-Toluidine	2000µg/ml in Purge & Trap Methanol	1ml
REAZO035	Aniline	1000µg/ml in Purge & Trap Methanol	1ml
REAZO036	Aniline	2000µg/ml in Purge & Trap Methanol	1ml
REAZO037	p-phenylenediamine	1000µg/ml in Purge & Trap Methanol	1ml
REAZO038	p-phenylenediamine	2000µg/ml in Purge & Trap Methanol	1ml
REAZO039	2-Nitroalinine	1000µg/ml in Purge & Trap Methanol	1ml
REAZO040	2-Nitroalinine	2000µg/ml in Purge & Trap Methanol	1ml
REAZO041	3-Nitroalinine	1000µg/ml in Purge & Trap Methanol	1ml
REAZO042	3-Nitroalinine	2000µg/ml in Purge & Trap Methanol	1ml
REAZO043	4-Nitroalinine	1000µg/ml in Purge & Trap Methanol	1ml
REAZO044	4-Nitroalinine	2000µg/ml in Purge & Trap Methanol	1ml